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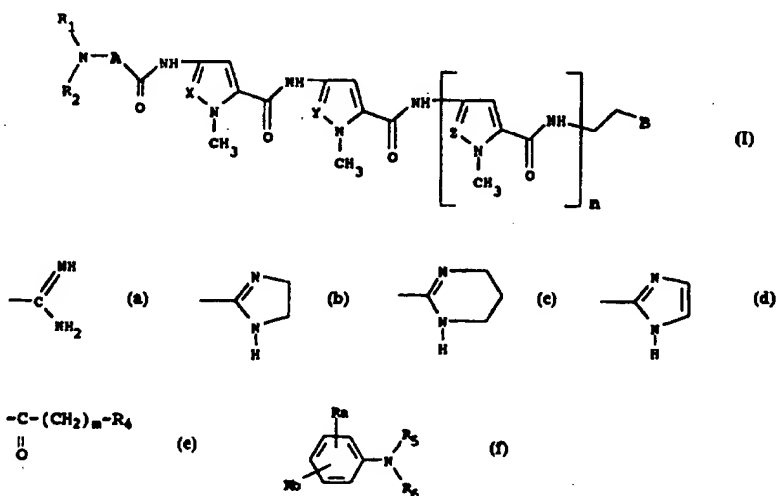
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(54) Title: DISTAMYCIN A ANALOGUES AS ANTITUMOUR OR ANTIVIRAL AGENTS



## (57) Abstract

The present invention relates to compounds of formula (I) wherein n is 0 or 1; each of X, Y, Z is independently N or CH; A is a pentatomic heteromonocyclic ring unsubstituted or substituted by a C<sub>1</sub>-C<sub>3</sub> alkyl group; B is (a); (b); (c) or (d); and either R<sub>1</sub> and R<sub>2</sub> are the same and they are both a C<sub>1</sub>-C<sub>6</sub> alkyl group unsubstituted or substituted by halogen or hydroxy; or one of R<sub>1</sub> and R<sub>2</sub> is hydrogen and the other is a group (e) in which m is zero or an integer of 1 to 4 and R<sub>4</sub> is aziridinyl; cyclopropyl; a vinyl group unsubstituted or substituted in position 1 or 2 by halogen or C<sub>1</sub>-C<sub>3</sub> alkyl; an oxyranyl group unsubstituted or substituted in position 2 by a C<sub>1</sub>-C<sub>3</sub> alkyl or halogen; or a group (f) in which R<sub>5</sub> and R<sub>6</sub> are both a C<sub>1</sub>-C<sub>6</sub> alkyl group unsubstituted or substituted by a halogen or hydroxy; provided that: a) when each of X, Y, Z is CH, then A is not thiophene or pyrrole unsubstituted or substituted by a C<sub>1</sub>-C<sub>3</sub> alkyl group; and b) when n is 1, each of X, Y, Z is CH and R<sub>1</sub> and R<sub>2</sub> are the same and they are both 2-chloroethyl, then A is not 1-methylimidazole or thiazole; and the pharmaceutically acceptable salt thereof. The compounds of the invention are useful as antitumor or antiviral agents.

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## DISTAMYCIN A ANALOGUES AS ANTITUMOUR OR ANTIVIRAL AGENTS

5 The invention relates to new peptidic compounds analogous to Distamycin A, to a process for their preparation, to pharmaceutical compositions containing them and to their use as therapeutic agents.

Distamycin A is an antibiotic substance with antiviral and  
10 oncolytic properties, having a polypyrrole framework (Nature 203, 1064 (1964); J. Med. Chem. 32, 774-778 (1989)).

The peptidic compounds of the invention are analogous to Distamycin A wherein the pyrrole rings are substituted partially or completely by other heteromonocyclic rings.

15 Analogous of Distamycin A, Netropsin or Lexitropsin wherein one or more pyrrole rings are substituted by a 1,2,4-triazole ring are reported, for example, in Chem. Res. Toxicol. 4, 241-252 (1991) and Heterocycles Vol. 31, 1629 (1990).

Distamycin derivatives wherein a pyrrole ring is substituted by  
20 a thiazole ring are reported in Anti-Cancer Drug Design 5, 3-20 (1990) and J. Org. Chem. 55, 728-737 (1990).

Distamycin derivatives wherein a pyrrole ring is substituted by an imidazole ring are reported in Anti-Cancer Drug Design 8, 173-192 (1993) and J. Am. Chem. Soc. Vol. 114, 5911-5919  
25 (1992).

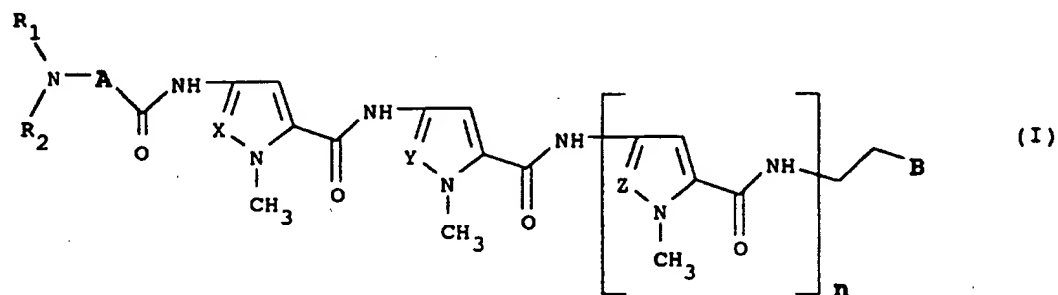
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Distamycin derivatives wherein a pyrrole ring is substituted by a pyrazole ring are reported in Anti-Cancer Drug Design 6, 501-517 (1991).

- 5 The present invention relates to compounds of the following formula (I)



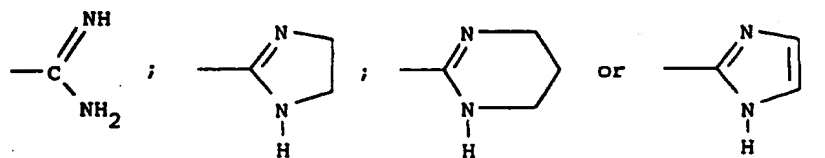
wherein

n is 0 or 1;

- 10 each of X, Y, Z is independently N or CH;

A is a pentatomic heteromonocyclic ring unsubstituted or substituted by a C<sub>1</sub>-C<sub>3</sub> alkyl group;

B is



- 15 and either R<sub>1</sub> and R<sub>2</sub> are the same and they are both a C<sub>1</sub>-C<sub>6</sub> alkyl group unsubstituted or substituted by halogen or hydroxy; or one of R<sub>1</sub> and R<sub>2</sub> is hydrogen and the other is a group



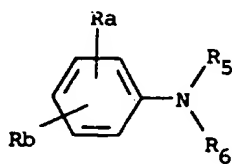
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in which m is zero or an integer of 1 to 4 and R<sub>4</sub> is aziridiny1; cyclopropyl; a vinyl group unsubstituted or substituted in position 1 or 2 by halogen or C<sub>1</sub>-C<sub>3</sub> alkyl; an oxyranyl group unsubstituted or substituted in position 2 by a C<sub>1</sub>-C<sub>3</sub> alkyl or halogen; or



a group in which R<sub>5</sub> and R<sub>6</sub> are both a C<sub>1</sub>-C<sub>6</sub> alkyl group unsubstituted or substituted by a halogen or hydroxy group, each of R<sub>a</sub> and R<sub>b</sub> independently is hydrogen, C<sub>1</sub>-C<sub>3</sub> alkyl or C<sub>1</sub>-C<sub>3</sub> alkoxy, provided that:

- 10 a) when each of X, Y, Z is CH, then A is not thiophene or pyrrole unsubstituted or substituted by a C<sub>1</sub>-C<sub>3</sub> alkyl group; and
- b) when n is 1, each of X, Y, Z is CH and R<sub>1</sub> and R<sub>2</sub> are the same and they are 2-chloroethyl, then A is not 1-methylimidazole or thiazole; and the pharmaceutically
- 15 acceptable salt thereof.

The invention includes also all the possible isomers covered by formula (I), both separately and in mixture.

The alkyl groups may be branched or straight chain.

- 20 A pentatomic heteromonocyclic ring is preferably a pentatomic saturated or unsaturated, most preferably unsaturated, heteromonocyclic ring containing one or two or three heteroatoms chosen from S or N.

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An unsubstituted pentatomic heteromonocyclic ring is, for example, thiophene, thiazole, imidazole, pyrrole, pyrazole, 1,2,4-triazole.

A pentatomic heteromonocyclic ring substituted by C<sub>1</sub>-C<sub>3</sub> alkyl is, for example, 1-methylpyrazole, 1-methyl-1,2,4- triazole, 1-methylpyrrole and 1-methylimidazole.

The imidazole and pyrrole rings are preferably linked in position 2 to the -C- group and in position 4 to the -NH-

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group.

The thiazole rings are preferably linked in position 2 to the -N- and in position 4 to the -CO- group.

The pyrazole and the 1,2,4-triazole rings are preferably linked  
15 in position 3 to the -N- group and in position 5 to the -CO- group.

A C<sub>1</sub>-C<sub>6</sub> alkyl group is preferably a C<sub>1</sub>-C<sub>3</sub> alkyl group, for example methyl, ethyl.

A halogen atom is preferably chlorine, bromine or fluorine.

20 When R<sub>1</sub> and R<sub>2</sub> are both a C<sub>1</sub>-C<sub>6</sub> alkyl group they are preferably a C<sub>1</sub>-C<sub>3</sub> alkyl group, for example methyl and ethyl.

When R<sub>1</sub> and R<sub>2</sub> are both a C<sub>1</sub>-C<sub>6</sub> alkyl group substituted by halogen they are preferably 2-chloroethyl, 2-bromoethyl, 2-fluoroethyl.

25 When R<sub>1</sub> and R<sub>2</sub> are both a C<sub>1</sub>-C<sub>6</sub> alkyl group substituted by hydroxy, they are preferably 2-hydroxyethyl.

When one of R<sub>1</sub> and R<sub>2</sub> is a group -C-(CH<sub>2</sub>)<sub>m</sub>-R<sub>4</sub> in which m is zero

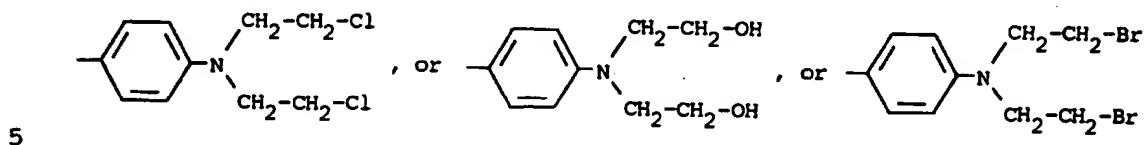
$$\begin{array}{c} \parallel \\ \text{O} \end{array}$$

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or an integer of 1 to 4,  $R_4$  is preferably aziridinyl, cyclopropyl,  $\alpha$ -bromovinyl,  $\alpha$ -chlorovinyl, 2-fluoroxyranyl, 2-methyloxyranyl,



Pharmaceutically acceptable salts of the compounds of formula (I) are their salts with pharmaceutically acceptable, either inorganic or organic, acids.

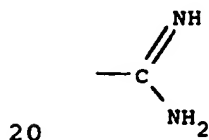
10 Examples of inorganic acids are hydrochloric, hydrobromic, sulfuric and nitric acid; examples of organic acids are acetic, propionic, succinic, malonic, citric, tartaric, methanesulfonic and p-toluenesulfonic acid.

15 A preferred class of compounds of the invention are the compounds of formula (I) wherein:

$n$  is 0 or 1; each of  $X$ ,  $Y$ ,  $Z$  independently is  $N$  or  $\text{CH}$ ;

$A$  is thiazole, imidazole, 1,2,4-triazole, 1-methyl-pyrrole, 1-methylimidazole or 1-methylpyrazole;

$B$  is



and either  $R_1$  and  $R_2$  are the same and they are both 2-chloroethyl, 2-fluoroethyl, 2-bromoethyl or one of  $R_1$  and  $R_2$  is hydrogen and the other is  $\alpha$ -bromoacryloyl, 4-N,N-bis(2-

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chloroethyl)amino-benzene-1-carbonyl, 4-N,N-bis(2-bromoethyl) amino-benzene-1-carbonyl, provided that:

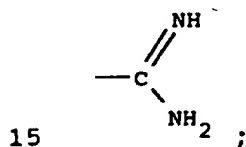
- a) when each of X, Y, Z is CH, then A is not 1-methyl-pyrrole; and
- 5 b) when n is 1, each of X, Y, Z is CH and R<sub>1</sub> and R<sub>2</sub> are the same and they are both 2-chloroethyl, then A is not 1-methylimidazole or thiazole, and the pharmaceutically acceptable salts thereof.

10 A particularly preferred class of compounds of the invention are the compounds of formula (I) wherein:

n is 0 or 1; each of X, Y, Z independently is N or CH;

A is 1-methyl-pyrrole or 1-methylpyrazole;

B is



and either R<sub>1</sub> and R<sub>2</sub> are the same and they are both 2-chloroethyl, 2-fluoroethyl, 2-bromoethyl or one of R<sub>1</sub> and R<sub>2</sub> is hydrogen and the other is α-bromoacryloyl, 4-N,N-bis(2-chloroethyl)amino-benzene-1-carbonyl, 4-N,N-bis(2-bromoethyl) amino-benzene-1-carbonyl.

20

Examples of specific compounds under this invention are the following compounds:

- 1) β-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-
- 25



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carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
propionamidine;

- 2)  $\beta$ -[1-methyl-4-[1-methyl-4-[2-[4-N,N-bis(2-chloroethyl)  
amino-benzene-1-carboxamido]thiazole-4-carboxamido]pyrrole-  
5 2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 3)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-  
chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]  
propionamidine;
- 10 4)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-  
chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]  
propionamidine;
- 15 5)  $\beta$ -[1-methyl-3-[1-methyl-4-[2-[4-N,N-bis(2-chloroethyl)  
amino-benzene-1-carboxamido]thiazole-4-carboxamido]pyrrole-  
2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 6)  $\beta$ -[1-methyl-4-[1-methyl-4-[3-[4-N,N-bis(2-chloroethyl)  
amino-benzene-1-carboxamido]-1,2,4-triazole-5-carboxamido]  
pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 20 7)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-  
chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-  
carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]  
propionamidine;
- 25 8)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-  
chloroethyl)amino-benzene-1-carboxamido]imidazole-2-

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carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
propionamidine;

- 9)  $\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 10)  $\beta$ -[1-methyl-4-[1-methyl-3-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]-1,2,4-triazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 11)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 12)  $\beta$ -[1-methyl-3-[1-methyl-3-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]-1,2,4-triazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 13)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 14)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;

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- 15)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 5 16)  $\beta$ -[1-methyl-4-[1-methyl-4-[2( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 17)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 10 18)  $\beta$ -[1-methyl-4-[1-methyl-4-[3-( $\alpha$ -bromoacrylamido)-1,2,4-triazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 19)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 15 20)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 20 21)  $\beta$ -[1-methyl-3-[1-methyl-4-[2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 22)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
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- 23)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 24)  $\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 25)  $\beta$ -[1-methyl-4-[1-methyl-3-[3-( $\alpha$ -bromoacrylamido)1,2,4-triazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 10 26)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 15 27)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-[1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 20 28)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 25 29)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

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- 30)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 5 31)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 32)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 10
- 33)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 15
- 34)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 20
- 35)  $\beta$ -[1-methyl-3-[1-methyl-4-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 25 36)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-

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- carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrazole-5-carboxamido]propionamidine;
- 37)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-  
bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-  
5 carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrazole-5-carboxamido]propionamidine;
- 38)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[3-[4-N,N-bis(2-  
chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
10 pyrazole-5-carboxamido]propionamidine;
- 39)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-  
bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrazole-5-carboxamido]propionamidine;
- 15 40)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[1-methyl-3-[4-N,N-  
bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-  
carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]  
pyrazole-5-carboxamido]propionamidine;
- 41)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-  
20 bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]  
pyrazole-5-carboxamido]propionamidine;
- 42)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-  
bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-  
25 carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]  
pyrazole-5-carboxamido]propionamidine;

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- 43)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 5 44)  $\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-4(2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine;
- 45)  $\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-4-(3-( $\alpha$ -bromoacrylamido)1,2,4, triazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine;
- 10
- 46)  $\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine;
- 15
- 47)  $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine;
- 48)  $\beta$ -(1-methyl-3-(1-methyl-3-(2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine;
- 20
- 49)  $\beta$ -(1-methyl-3-(1-methyl-3-(3-( $\alpha$ -bromoacrylamido)1,2,4, triazole-5-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine;

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- 50)  $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-4-(1-methyl-3-( $\alpha$ -  
bromoacrylamido)pyrazole-5-carboxamido)pyrrole-2-  
carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)  
propionamidine;
- 5 51)  $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-4-(1-methyl-4-( $\alpha$ -  
bromoacrylamido)imidazole-2-carboxamido)pyrrole-2-  
carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)  
propionamidine;
- 52)  $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-4-(1-methyl-4-( $\alpha$ -  
10 bromoacrylamido)pyrrole-2-carboxamido)pyrrole-2-  
carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)  
propionamidine;
- 53)  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-3-( $\alpha$ -bromoacrylamido)  
pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrazole-5-  
15 carboxamido)propionamidine;
- 54)  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)  
imidazole-2-carboxamido)pyrrole-2-carboxamido)pyrazole-5-  
carboxamido)propionamidine;
- 55)  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-(1-methyl-3-( $\alpha$ -  
20 bromoacrylamido)pyrazole-5-carboxamido)pyrrole-2-  
carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)  
propionamidine;
- 56)  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-(1-methyl-4-( $\alpha$ -  
25 bromoacrylamido)imidazole-2-carboxamido)pyrrole-2-  
carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)  
propionamidine;



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- 57)  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-(3-( $\alpha$ -  
bromoacrylamido)1,2,4, triazole-5-carboxamido)pyrrole-2-  
carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)  
propionamidine;
- 5 58)  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-(1-methyl-4-( $\alpha$ -  
bromoacrylamido)pyrrole-2-carboxamido)pyrrole-2-  
carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)  
propionamidine;
- 59)  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-3-(1-methyl-3-( $\alpha$ -  
10 bromoacrylamido)pyrazole-5-carboxamido)pyrazole-5-  
carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)  
propionamidine;
- 60)  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -  
15 bromoacrylamido)pyrrole-2-carboxamido)pyrazole-5-  
carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)  
propionamidine;
- 61)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[2-[4-N,N-bis  
(2-chloroethyl)amino-benzene-1-carboxamido]thiazole-4-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
20 pyrrole-2-carboxamido]propionamidine;
- 62)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-  
bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrrole-2-carboxamido]propionamidine;
- 25 63)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-[4-N,N-bis

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- (2-chloroethyl)amino-benzene-1 carboxamido]1,2,4,  
triazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-  
carboxamido]pyrrole-2-carboxamido]propionamidine;
- 64)  $\beta$ -[1-methyl-3-[1-methyl-3-[2-[4-N,N-bis(2-chloroethyl)  
5 amino-benzene-1-carboxamido]thiazole-4-carboxamido]  
pyrazole-5-carboxamido]pyrazole-5-carboxamido]  
propionamidine;
- 65)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-  
10 bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-  
carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]  
pyrrole-2-carboxamido]propionamidine;
- 66)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-  
bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]  
15 pyrrole-2-carboxamido]propionamidine;
- 67)  $\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-  
chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-  
carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]  
propionamidine;
- 20 68)  $\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-  
chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]  
propionamidine;
- 69)  $\beta$ -(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)  
25 pyrrole-2-carboxamido)pyrazole-5-carboxamido)pyrrole-2-  
carboxamido)propionamidine;

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- 70)  $\beta$ -(1-methyl-4-(1-methyl-3-(1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)propionamidine;
- 5 71)  $\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine;
- 10 72)  $\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine;
- 15 73)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 20 74)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 25 75)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 76)  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-

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carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrrole-2-carboxamido]propionamidine;

77)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-3-[4-N,N-  
bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-  
5 carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]  
pyrazole-5-carboxamido]propionamidine;

78)  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-  
bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]  
10 pyrazole-5-carboxamido]propionamidine;

79)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-  
bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrazole-5-carboxamido]propionamidine;

80)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-  
bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrazole-5-carboxamido]propionamidine;

81)  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-  
20 bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]  
pyrazole-5-carboxamido]propionamidine, and the  
pharmaceutically acceptable salts thereof, especially the  
salts with hydrochloric acid or hydrobromic acid.

25

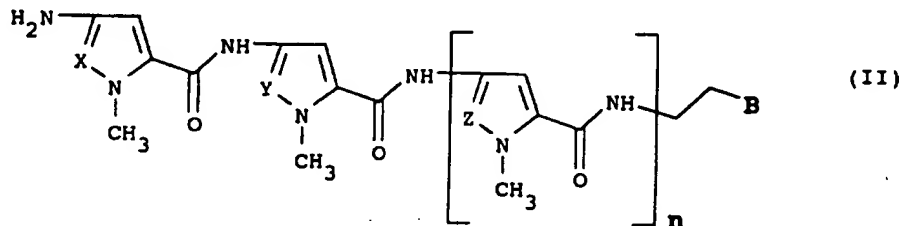
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The compounds of formula (I) or a pharmaceutically acceptable salt thereof are prepared by a process comprising:

a) reacting a compound of formula (II)

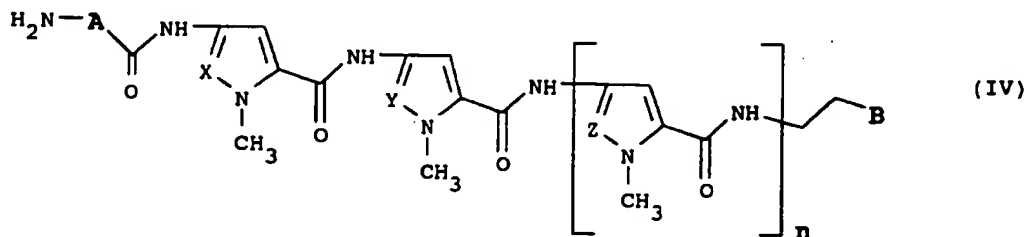


wherein X, Y, Z, B and n are as defined above or a salt thereof with a compound of formula (III)

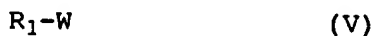


wherein R<sub>1</sub>, R<sub>2</sub> and A are as defined above and W is hydroxy or a leaving group; or

b) reacting a compound of formula (IV)



wherein A, X, Y, Z, B and n are as defined above or a salt thereof with a compound of formula (V)



wherein R<sub>1</sub> and W are as defined above so obtaining a compound of formula (I) wherein R<sub>1</sub> is as defined above and R<sub>2</sub> is hydrogen or is the same as R<sub>1</sub> and, if desired, salifying a

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compound of formula (I) or obtaining a free compound from a salt, and/or, if desired, separating a mixture of isomers of formula (I) into the single isomers.

The leaving group W in the compounds (III) and (V) may be, for example, halogen, in particular chlorine, or another displaceable group such as, for instance 2,4,5-trichlorophenoxy, 2,4-dinitrophenoxy, succinamido-N-oxy, imidazolyl, pivaloyloxy [ $-\text{O}-\text{C}-\text{C}(\text{CH}_3)_3$ ], ethyloxy formate

( $-\text{O}-\text{C}-\text{OEt}$ ), isopropoxyformate [ $-\text{O}-\text{C}-\text{O}-\text{CH}(\text{CH}_3)_3$ ] or  $\alpha$ -bromoacryloxy [ $-\text{OC}-\text{C}=\text{CH}_2$ ].

The reaction between a compound of formula (II), according to the process variant a), and a compound of formula (III) wherein W is OH, is preferably carried out in a molar ratio ranging from about 1:1 to about 1:2 in an organic solvent such as, e.g., dimethylsulphoxide, hexamethylphosphotriamide, dimethylacetamide, dioxane, or, preferably, dimethylformamide, or their aqueous mixtures, in the presence of an organic base such as, e.g., triethylamine or N,N'-diisopropylethylamine or an inorganic base such as, e.g., sodium bicarbonate and in the presence of a condensing agent such as, e.g., N,N'-dicyclohexylcarbodiimide, or preferably 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride.

The reaction temperature may vary from about -10°C to about 50°C and the reaction time from about 1 to about 24 hours.

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The reaction between a compound of formula (II) and a compound of formula (III), wherein W is a leaving group, as defined above, according to the process step a), may be carried out at a molar ratio ranging from about 1:1 to about 1:2 in an organic solvent such as, e.g., dimethylformamide, dioxane, pyridine, tetrahydrofuran or their aqueous mixture in the presence of an organic base, e.g. N,N'-diisopropylethylamine, triethylamine, or an inorganic base, e.g. sodium bicarbonate, at a temperature from about 0°C to about 100°C and for a time varying from about 2 hours to about 48 hours.

The reaction between a compound of formula (IV) and a compound of formula (V), according to the process variant b), may be carried out in analogous conditions as reported hereabove for the process variant a).

When W in the compounds of formula (V) is a leaving group, it is preferably chlorine.

When R<sub>1</sub> and R<sub>2</sub> have the same meaning, the reaction is carried out in a molar ratio, between (V) and (IV), from about 2:1 to about 3:1 in an organic solvent or their aqueous mixtures in presence of an organic or inorganic base as reported hereabove. The salification of a compound of formula (I) as well as the preparation of a free compound from a salt and the separation of a mixture of isomers into the single isomers may be carried out by known standard methods.

The compounds of formula (I) prepared according to the above described procedures may be purified by conventional methods

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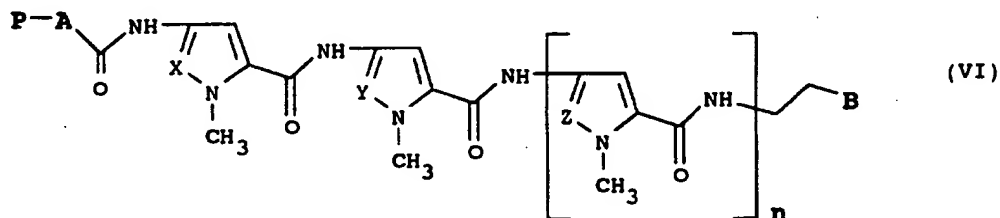
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such as, e.g., H.P.L.C. silica gel or alumina column chromatography, and/or by recrystallization from an organic solvent such as, e.g., a lower aliphatic alcohol, e.g. methyl, ethyl, propyl, isopropyl alcohol or dimethylformamide.

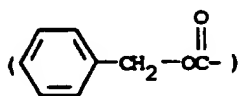
- 5 The compounds of formulae (III) and (V) are known compounds or may be prepared by standard methods from known compounds, as described for example in J.O.C. 26, 4996 (1961); J.A.C.S. 62, 3495 (1940); J. Med. Chem. 9, 882 (1966), 25, 178 (1982), 21, 16 (1978) and 8, 167 (1965).

10

The compounds of formula (IV) may be prepared from compounds of formula (VI)



- 15 wherein P is a nitro group or an amino group appropriately protected with a group such as t-butyloxycarbonyl  $((\text{CH}_3)_3\text{CO}-\overset{\text{O}}{\parallel}{\text{C}}-)$ , triphenylmethyl  $[(\text{Ph})_3\text{C}-]$  or preferably carbobenzyloxy,



20

, and A, X, Y, Z, n, B are as defined above.

- The conversion of the nitro group into an amino group may be carried out according to known procedures using for instance a solvent such as dioxane, methanol, ethanol or their aqueous mixture at room temperature and under 50 psi pressure of  
25 hydrogen in the presence of 10% Palladium on charcoal.



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The conversion of protected amine into the free amine may be carried out according to known procedures.

See for example J. Org. Chem. 43, 2285, (1978) when the protecting group is t-butyloxycarbonyl; J. Org. Chem. 44, 811  
5 (1979); J. Am. Chem. Soc. 78, 1359 (1956) when the protecting group is triphenylmethyl or Ber. 65, 1192 (1932) when the protecting group is carbobenzyloxy.

The compounds of formula (VI) may be prepared by a process  
10 comprising reacting a compound of formula (II) with a compound of formula (VII)



15 wherein A, W and P are as defined above, W being preferably chlorine or OH.

The reaction conditions are the same as reported above for the process variant (a).

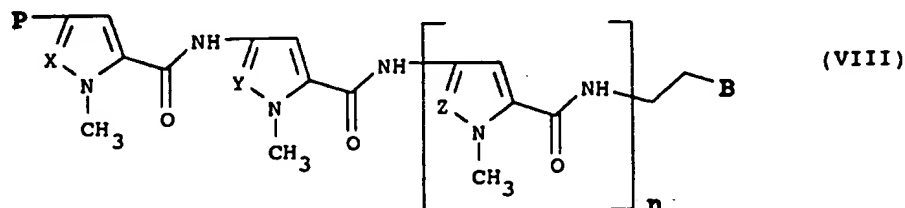
The compounds of formula (VII) are known or may be prepared by  
20 known methods. See for example: Acta. Chem. Scan. 44, 75 (1990); Tetrahedron 44, 5833 (1988); J. Org. Chem. 50, 3774 (1985) and 52, 3493 (1987); Heterocycles 31, 1629 (1990).

The compounds of formula (II) may be prepared from compounds of  
25 formula (VIII)

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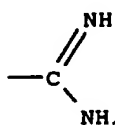


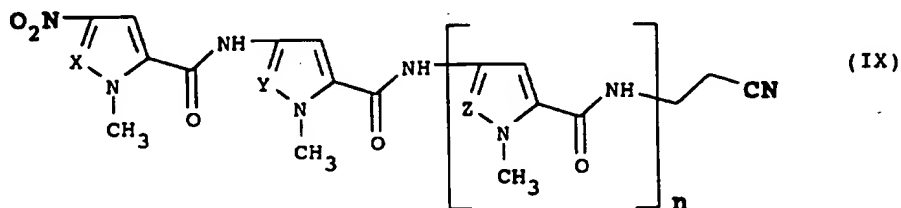
wherein P, X, Y, Z, n and B are as defined above and the reaction conditions for transformation of the P group into amino group are the same as reported above for the conversion  
 5 of the compounds of formula (VI) into the compounds of formula (IV).

The compounds of formula (VIII) may be prepared by different methods depending on the meaning of P and B.

10

The compounds of formula (VIII), wherein X, Y, Z and n

are as defined above, P is a nitro group and B is  may be prepared from compounds of formula (IX)



15 wherein X, Y, Z and n are as defined above.

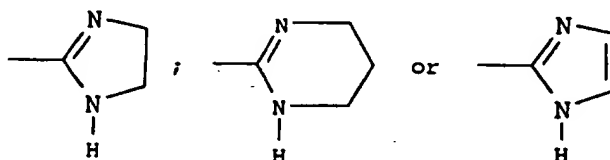
The conversion of the nitrile group into the amidine may be carried out using Pinner Synthesis as reported in Ann. Chim. (Paris) 14, 5, 23-27 (1970).

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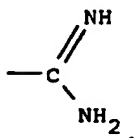
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The compounds of formula (VIII), wherein X, Y, Z and n are as defined above, P is a nitro group and B is a group



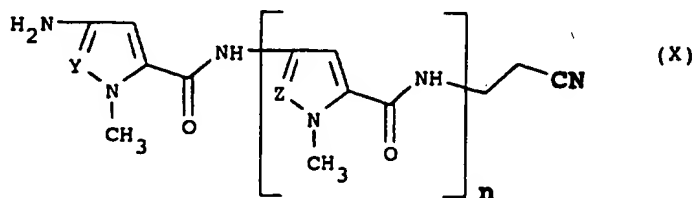
may be obtained from the compounds of formula (VIII) wherein X,  
 5 Y, Z and n are as defined above, P is a nitro group and B is



The conversion of the amidino group into the 2-imidazole ring  
 may be, e.g., carried out by reaction with, e.g.,  
 10 aminoacetaldehydedimethylacetal according to known procedure,  
 while ethylenediamine may be used for converting the amidino  
 into the 2-imidazoline group; the conversions into the other  
 heterocycles may be carried out in similar way by known  
 methods.

15

The compounds of formula (IX) may be prepared by a process  
 comprising reacting a compound of formula (X)

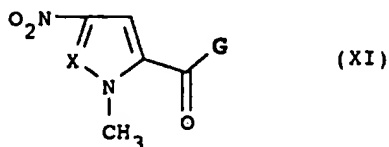


wherein Y, Z and n are as defined above with compounds of  
 20 formula (XI)

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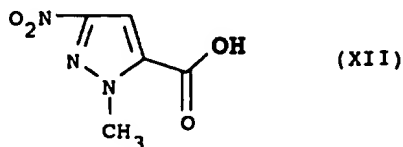
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wherein X is as defined above and G is a leaving group such as chlorine and imidazolyl or an OH group. The reaction conditions are the same reported in the process steps (a).

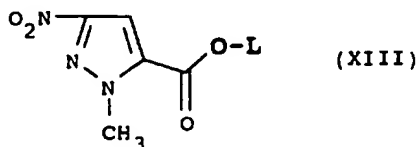
- 5 The compounds of formula (XI) wherein X is CH are known or may be prepared by standard methods, see J. Med. Chem. 26, 1042-1049 (1983).

The compound of formula (XI) wherein X is N may be prepared  
10 from compounds of formula (XII)



The reaction may be carried out according to known procedure using, for instance, when G is chlorine, SOCl<sub>2</sub> in an inert solvent such as dioxane, benzene, at reflux temperature for a  
15 time from about 0.5 to about 2 hours; when G is imidazolyl, carbonyldiimidazole can be used and a solvent such as benzene, ethyl acetate, dioxane or dimethylformamide.

The compounds of formula (XII) may be prepared from compounds  
20 of formula (XIII)



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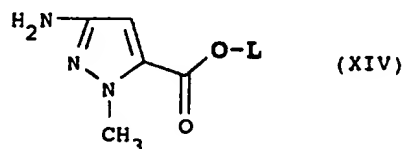
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wherein L is an alkyl group such as methyl or ethyl.

The reaction may be carried out by hydrolytic condition according to known procedures using for instance NaOH, KOH in an organic solvent such as dioxane or acetonitrile, at room temperature for a time from about 1 to about 24 hours.

The compound of formula (XIII) may be prepared from a compound of formula (XIV)



wherein L is as defined above, by oxidation.

The reaction may be carried out using from about 3 equivalents to about 5 equivalents of 3-chloroperbenzoic acid in an organic solvent such as chloroform, dioxane or acetonitrile at a temperature from about 50°C to about 80°C and for a time from about 1 to about 5 hours.

The compounds of formula (XIV) are known or may be prepared by known methods, see for example Acta Chem. Scan. 44, 75 (1990) or J. Org. Chem. 54, 431-434 (1989).

The compounds of formula (X), wherein n is 0 or 1 and Y=Z=CH, are known, see for example J. Med. Chem. 26, 1042-1049 (1983) and J. Org. Chem. 50, 3774 (1985) and 52, 3493 (1987).

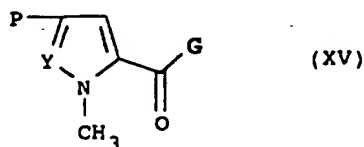
The compounds of formula (X), wherein n is 0 or 1 and Y and Z are N, may be prepared by known methods, see for example Anti-Cancer Drug Des. 6, 501-517 (1991).

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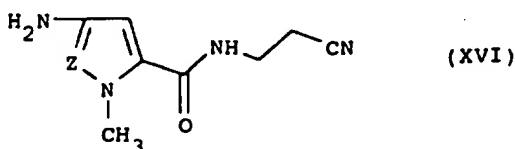
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The compounds of formula (X) wherein n is 1 and one of Y and Z is N and the other is CH, may be prepared reacting a compound of formula (XV)



- 5 wherein P, Y, G are as defined above with a compound of formula (XVI)



The reaction may be carried out in the same condition as reported in the process step (a).

- 10 The compounds of formula (XV) are known or may be prepared by known methods, for example, when P is NO<sub>2</sub> and Y is N the reaction may be carried out as reported above for the compound (XI).

- The compounds of formula (XVI) are known compounds (J. Org. Chem. 50, 3774 (1985) and 52,3493 (1987); Anti-Cancer Drug Des. 6, 501-517 (1991)).
- 15

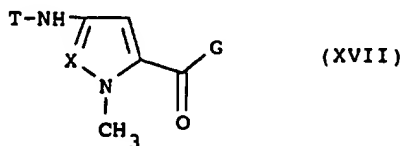
The compounds of formula (VIII) wherein P is an amino group protected as tertbutyloxycarbonyl (Boc), triphenylmethyl or, preferably, carbobenzyloxy (Cbz), and X, Y, Z, n, B are as defined above, may be prepared by a process comprising reacting a compound of formula (XVII)

20

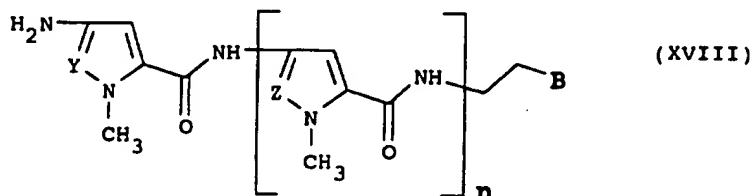
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wherein T is a protecting group of the amino group, preferably carbobenzyloxy, and G is as defined above, with a compound of formula (XVIII)

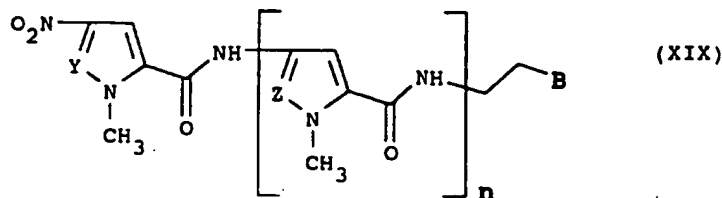


wherein Y, Z, n and B are as defined above.

The reaction conditions are the same as reported in the process steps (a).

The compounds of formula (XVII) are known or may be prepared by known methods, see for example Acta Chem. Scan. 44, 75-81 (1990) and J. Med. Chem. 26, 1042-1049 (1983).

The compounds of formula (XVIII) may be prepared by reducing compounds of formula (XIX)



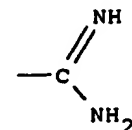
wherein Y, Z, n and B are as defined above.

The reduction may be carried out by catalytic hydrogenation according to known procedures.

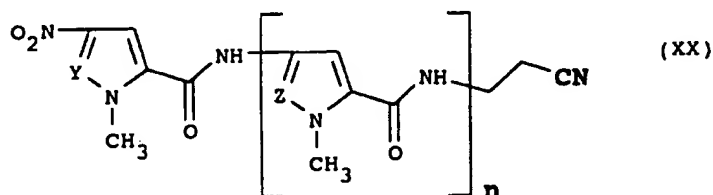
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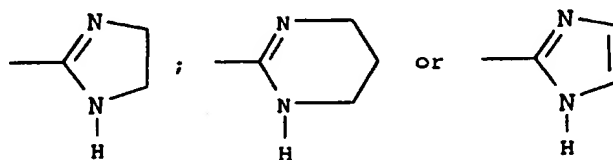
The compound of formula (XIX), wherein B is  $\text{---C(=NH)NH}_2$  and Y, Z, n are as defined above, may be prepared from compounds of formula (XX)



5 wherein Y, Z and n are as defined above.

The conversion of the nitrile group into the amidine may be carried out using Pinner synthesis as reported in Ann. Chim. (Paris) 14, 5, 23-27 (1970).

The compounds of formula (IX) wherein B is a group



10

and Y, Z, n are as defined above, may be prepared from amidine derivatives using procedure as reported above for the corresponding compounds of formula (VIII).

The compounds of formula (XX) wherein n is as defined above and  
15 each of Y and Z is  $\text{---CH---}$  are known compounds, see for example J. Org. Chem. 50, 3774 (1985) and 52, 3493 (1987).

The compounds of formula (XX) wherein n is 0 and Y is N may be prepared by a process comprising reacting a compound of formula (XII) as defined above with 3-amino propionitrile.



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The reaction may be carried out according to known procedures, see for example Anti-Cancer Drug Des. 6, 501-517 (1991).

The compounds of formula (XX), wherein n is 1 and Y and Z are as defined above but not both CH, may be prepared by a process  
5 comprising reacting a compound of formula (XI), wherein X is N or CH and G is as defined above, with a compound of formula (XVI) as defined above.

The reaction conditions are the same as reported above in the process step (a).

10 The compounds of the invention show cytotoxic properties towards tumor cells so that they can be useful as antineoplastic agents, e.g. to inhibit the growth of various tumors such as, for instance, carcinomas, e.g. mammary carcinoma, lung carcinoma, bladder carcinoma, colon carcinoma,  
15 ovary and endometrial tumors. Other neoplasias in which the compounds of the invention could find application are, for instance, sarcomas, e.g. soft tissue and bone sarcomas, and the hematological malignancies such as, e.g., leukemias.

20 The antitumor activity was evaluated in vitro by cytotoxicity studies carried out on murine L1210 leukemia cell. Cells were derived from in vivo tumors and established in cell culture. Cells were used until the tenth passage. Cytotoxicity was determined by counting surviving cells after 4 hours treatment  
25 and 48 hours growth in drug-free medium.

The percentage of cell growth in the treated cultures was compared with that of controls. ID<sub>50</sub> values (doses inhibiting

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50% of the cellular growth in respect to controls) were calculated on dose-response curves.

Thus, for example, the compound of the invention  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine hydrochloride showed an ID<sub>50</sub> of 0.5  $\mu$ g/ml.

The compounds of the invention were tested also in vivo on murine L<sub>1210</sub> leukemia and on murine reticulosarcoma M 5076, showing a very good antitumoral activity, with the following procedure.

L<sub>1210</sub> murine leukemia was maintained in vivo by i.p. serial transplantation. For experiments, 10<sup>5</sup> cells were injected i.p. in CD2F1 female mice, obtained from Charles River Italy. Animals were 8 to 10 weeks old at the beginning of the experiments. Compounds were administered i.p. at day +1 after tumor cells injections.

M5076 reticulosarcoma was maintained in vivo by i.m. serial transplantation. For experiments, 5x10<sup>5</sup> cells were injected i.m. in C57B16 female mice, obtained from Charles River Italy. Animals were 8 to 10 weeks old at the beginning of the experiments. Compounds were administered i.v. at day 3, 7 and 11 after tumor injection.

Survival time of mice and tumor growth were calculated and activity was expressed in term of T/C% and T.I.%.

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$$\text{T/C} = \frac{\text{median survival time treated group}}{\text{median survival time untreated group}} \times 100$$

5 T.I. = % inhibition of tumor growth respect to control

Tox: number of mice which died for toxicity.

Tox determination was made when mice died before the control and/or tested significant body weight loss and/or spleen and/or  
10 liver size reduction were observed.

The compounds of the invention showed a high antitumor activity in these tumor models.

For example, the compound  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-  
15 2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido] propionamidine hydrochloride (FCE 28164) was tested against intraperitoneal L<sub>1210</sub> murine leukemia showing the activity reported in the table below.

	mg/kg	T/C %	Tox
20 Compound FCE 28164	25	259	0/10

The compounds of the invention can also be useful as antiviral agents.

25 The compounds of the invention can be administered by the usual routes, for example, parenterally, e.g. by intravenous

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injection or infusion, intramuscularly, subcutaneously, topically or orally.

The dosage depends on the age, weight and conditions of the patient and on the administration route.

5 For example, a suitable dosage for administration to adult humans may range from about 0.05 to about 100 mg pro dose 1-4 times a day.

The pharmaceutical compositions of the invention contain a compound of formula (I) as the active substance, in association  
10 with one or more pharmaceutically acceptable excipients.

The pharmaceutical compositions of the invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form.

For instance, solutions for intravenous injection or infusion  
15 may contain as carrier, for example, sterile water or preferably, they may be in the form of sterile aqueous isotonic saline solutions.

Suspensions or solutions for intramuscular injections may contain, together with the active compound a pharmaceutically  
20 acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

In the form for topical application, e.g. creams, lotions or pastes for use in dermatological treatment, the active  
25 ingredient may be mixed with conventional oleaginous or emulsifying excipients.

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The solid oral forms, e.g. tablets and capsules, may contain, together with the active compound, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch and potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents, e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethyl-cellulose, polyvinylpyrrolidone; disaggregating agents, e.g. a starch, alginic acid, alginates, sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, for instance, lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Said pharmaceutical preparations may be manufactured in a known manner, for example by means of mixing, granulating, tableting, sugar-coating, or film-coating processes.

Furthermore, according to the invention there is provided a method of treating viral infections and tumors in a patient in need of it, comprising administering to the said patient a composition of the invention.

The following examples illustrate but do not limit the invention.

The abbreviations DMF and THF stand, respectively, for dimethylformamide and tetrahydrofuran.

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Example 1

$\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride

5 (Compound no. 1)

*Step one* - Synthesis of the intermediate 1-methyl-3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrazole-5-carboxylic acid.

10

To a solution containing 0.84 g of methyl 1-methyl-3-aminopyrazole-5-carboxylate in 20 ml of dioxane (prepared as in J.Org.Chem. 54, 431-434, 1989) and 0.76 ml of triethylamine, 1.42 g of 4-N,N-bis(2-chloroethyl) aminobenzoyl chloride  
15 (prepared as in J.Org.Chem. 26, 4996-4997, 1961) were added. The mixture was stirred overnight, concentrated to small volume, diluted with 30 ml of 5% hydrochloric acid and extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulphate, after evaporation of the solvent a  
20 solid residue was obtained which was purified by recrystallization from diethyl ether to yield 1.64 g of methyl 1-methyl-3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrazole-5-carboxylate.

The derivative obtained (1.64 g) was dissolved in 15 ml of  
25 dioxane and added of 4.1 ml of 2 N potassium hydroxide.

The mixture was stirred for two hours, concentrated to small volume, acidified with 10% hydrochloric acid and extracted with

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ethyl acetate. The organic phase was dried over anhydrous sodium sulphate, the solvent evaporated in vacuo and the solid residue purified by recrystallization from ethyle acetate-hexane yielding 1.07 g of the intermediate.

5

m.p. 129-130°C

PMR(DMSO-d<sub>6</sub>)  $\delta$  : 13.25 (b.s., 1H), 10.68 (s, 1H), 7.92 (d, J= 8.7Hz, 2H), 7.13 (s, 1H), 6.82 (d, J= 8.7Hz, 2H), 4.03 (s, 3H), 3.78 (m, 4H), 2.50 (m, 4H)

10

By analogous procedure the following compounds can be prepared:

2-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]  
thiazole-4-carboxylic acid

m.p. 270-275°C dec.

15 PMR(DMSO-d<sub>6</sub>)  $\delta$  : 12.82 (b.s., 1H), 12.64 (b.s., 1H), 8.04 (d, J= 8.7Hz, 2H), 7.98 (s, 1H), 6.88 (d, J= 8.7Hz, 2H), 3.79 (m, 8H)

3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]  
1,2,4, triazole-5-carboxylic acid;

20 1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-  
carboxamido]pyrrole-2-carboxylic acid

m.p. 155-157°C

PMR(DMSO-d<sub>6</sub>)  $\delta$  : 11.75 (b.s., 1H), 9.91 (s, 1H), 7.81 (d, J= 8.7Hz, 1H), 7.28 (d, J= 1.7Hz, 1H), 6.88 (d, J= 8.7Hz, 1H), 6.64  
25 (d, J= 1.7Hz, 1H), 3.83 (s, 1H), 3.35 (m, 8H)

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1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxylic acid.

Step two - The title compound

5

To a solution of 0.25 g of intermediate in 20 ml of dry dimethylformamide was added 0.24 g of  $\beta$ -[1-methyl-4-[1-methyl-4-aminopyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine, dihydrochloride (prepared as in J.Med.Chem.32, 774-778, 1989), 0.13 ml of N,N'-diisopropylethylamine and 0.17 g of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride.

The mixture was stirred overnight at room temperature and brought to pH 4-5 with 10% hydrochloric acid.

15 After evaporation of the solvent a solid residue was obtained which was purified by HPLC using as eluant a mixture of acetonitrile-water-trifluoroacetic acid (10-90-0.1), yielding 0.204 g of the title compound.

20 FAB-MS: m/z 698, (41, [M+H]<sup>+</sup>); 244 (100)

m.p. 210-215°C

PMR(DMSO-d<sub>6</sub>)  $\delta$ : 10.65 (s, 1H), 10.47 (s, 1H), 9.98 (s, 1H), 9.0 (b.s., 2H), 8.6 (b.s., 2H), 8.22 (m, 1H), 7.93 (m, 2H), 7.45 (s, 1H), 7.30 (d, J= 1.7Hz, 1H), 7.19 (d, J= 1.7Hz, 1H), 7.09 (d, J= 1.7Hz, 1H), 6.94 (d, J= 1.7Hz, 1H), 6.8 (m, 2H), 4.04 (s, 3H), 3.85 (s, 3H), 3.8 (s, 3H), 3.6-3.9 (m, 8H), 3.49 (m, 2H), 2.6 (m, 2H)



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By analogous procedure and using the opportune starting material the following compounds can be prepared:

- 5  $\beta$ -[1-methyl-4-[1-methyl-4-[2-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]thiazole-4-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride (Compound no. 2)

FAB-MS: m/z 701, (60, [M+H]<sup>+</sup>)

m.p. 230-233°C

- PMR(DMSO-d<sub>6</sub>)  $\delta$ : 9.91 (s, 1H), 9.78 (s, 1H), 8.87 (b.s., 2H), 8.55  
10 (b.s., 2H), 8.19 (m, 1H), 8.02 (m, 2H), 7.83 (b.s., 1H), 7.28 (d, J= 1.7Hz, 1H), 7.18 (d, J= 1.7Hz, 1H), 7.09 (d, J= 1.7Hz, 1H), 6.94 (d, J= 1.7Hz, 1H), 6.86 (m, 2H), 3.7-3.9 (m, 8H), 3.86 (s, 3H), 3.81 (s, 3H), 3.50 (m, 2H), 2.61 (m, 2H)

- 15  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride (Compound no. 8);

- $\beta$ -[1-methyl-4-[1-methyl-4-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride  
20 (Compound no. 6);

- $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrobromide  
25 (Compound no. 73);

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- $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine hydrobromide (Compound no. 77);
- 5  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine hydrobromide (Compound no. 78).

10 Example 2

- $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride (Compound
- 15 no. 13)

To a solution containing 0.3 g of N-deformyl distamycin A dihydrochloride(prepared as in J.Med. Chem. 32, 774-778, 1989) and 0.23 g of 1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxylic acid (prepared as

20 reported in step one of Example 1) in 10 ml of dry dimethylformamide was added 0.17 g of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride and 0.1 ml of N,N'-diisopropylethylamine.

- 25 The solution was stirred at room temperature overnight, the solvent evaporated in vacuo and the solid residue purified by HPLC using as eluant a mixture of acetonitrile-water-

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trifluoroacetic acid (10-90-0.1), yielding 0.25 g of the title compound.

FAB-MS: m/z 820, (20, [M+H]<sup>+</sup>)

5 U.V. (EtOH 95%):  $\lambda$  max 312.85,  $\epsilon$ =67791

PMR(DMSO-d<sub>6</sub>)  $\delta$ : 10.65 (b.s., 1H), 10.47 (b.s., 1H), 10.01 (s, 1H), 9.93 (s, 1H), 8.8 (b.s., 4H), 8.22 (m, 1H), 7.93 (m, 2H), 7.46 (s, 1H), 7.31 (d, J= 1.8Hz, 1H), 7.24 (d, J= 1.8Hz, 1H), 7.18 (d, J= 1.8Hz, 1H), 7.11 (d, J= 1.8Hz, 1H), 7.06 (d, J= 1.8Hz, 1H), 6.94 (d, J= 1.8Hz, 1H), 6.81 (m, 2H), 4.05 (s, 3H), 3.6-3.9 (m, 8H), 3.86 (s, 3H), 3.84 (s, 3H), 3.8 (s, 3H), 3.48 (m, 2H), 2.59 (m, 2H)

By analogous procedure and using the opportune starting material the following compounds can be prepared:

15  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[2-[4-N,N-bis (2-chloroethyl)amino-benzene-1-carboxamido]thiazole-4-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride (Compound no. 61);

20

$\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis (2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride

25 (Compound no. 62)

FAB-MS: m/z 820, (10, [M+H]<sup>+</sup>); 244

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U.V. (EtOH 95%):  $\lambda$  max 312.85,  $\epsilon=67791$

PMR(DMSO- $d_6$ )  $\delta$ : 10.41 (s,1H), 9.98 (s,1H), 9.96 (s,1H), 9.91 (s,1H), 8.8 (bs,4H), 8.22 (t,J=6 Hz,1H), 7.9 (m,2H), 7.58 (s,1H), 7.27 (d,J=1.8 Hz,1H), 7.23 (d,J=1.8 Hz,1H), 7.18 (d,J=1.8 Hz,1H), 7.16 (d,J=1.8 Hz,1H), 7.06 (d,J=1.8 Hz,1H), 6.94 (d,J=1.8 Hz,1H), 6.8 (m,2H), 3.98 (s,3H), 3.85 (s,3H), 3.83 (s,3H), 3.8 (s,3H), 3.6-3.9 (m,8H), 3.49 (m,2H), 2.60 (t,J=6.4 Hz,1H).

- 10  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine,hydrochloride (Compound no. 63);
- 15  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine,hydrobromide (Compound no. 76).

20 **Example 3**

$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine,hydrochloride (Compound no. 4)

25

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*Step one* - Synthesis of the intermediate  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-nitropyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionitrile.

- 5 To a solution containing 1 g of 1-methyl-4-nitropyrrole-2-carboxylic acid (prepared as reported in Tetrahedron 34, 2389, 1978) in 5 ml of dry benzene was added 3 ml of thionyl chloride. The mixture was refluxed for one hour, the solvent evaporated in vacuo, the solid residue was dissolved in 10 ml  
10 of dioxane and the solution was added to a solution of 1.86 g of  $\beta$ -[1-methyl-3-[1-methyl-3-aminopyrazole-5-carboxamido]pyrazole-5-carboxamido]propionitrile (prepared as reported in Anti Cancer Drug Des.6,501-517,1991) and 0.82 ml triethylamine in 10 ml of dioxane.
- 15 The mixture was stirred at room temperature overnight, concentrated to small volume, acidified by 10% hydrochloric acid to yield a precipitate which was filtrated and purified by recrystallization from ethyl acetate-dioxane, yielding 1.92 g of intermediate.

20

m.p. 288-290°C

PMR(DMSO-d<sub>6</sub>)  $\delta$ : 11.2 (s,1H), 10.98 (s,1H), 8.99 (b.s.,1H), 8.23 (d,J= 1.7Hz,1H), 7.81 (d,J= 1.7Hz,1H), 7.57 (s,1H), 7.33 (s,1H), 4.06 (s,3H), 4.02 (s,3H), 3.97 (s,3H), 3.45 (t,J= 25 6Hz,2H), 2.78 (t,J= 6Hz,2H)

By analogous procedure the following compounds can be prepared:

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$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-nitroimidazole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionitrile;

$\beta$ -[1-methyl-3-[1-methyl-3-[2-nitrothiazole-4-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionitrile;

$\beta$ -[1-methyl-3-[1-methyl-3-[3-nitro-1,2,4-triazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionitrile.

10 *Step two* - Synthesis of the intermediate  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-aminopyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, dihydrochloride.

15 0.264 g of intermediate was suspended in anhydrous ethanol and the solution saturated with dry HCl gas. After 24 h at room temperature, the solvent was evaporated in vacuo and the residue treated with ammonia in ethanol. After 24 h at room temperature the solvent was evaporated in vacuo and the solid  
20 residue purified by recrystallization from methanol-ethyl acetate to yield 0.23 g of nitro derivative.

The nitro derivative (0.23 g) was dissolved in a mixture of methanol-dioxane-water-5% hydrochloric acid (10:5:5:1) and reduced over Pd catalyst (10% on charcoal) under hydrogen  
25 pressure (50 psi) in a Parr apparatus. The solution obtained

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after filtration of the catalyst was evaporated in vacuo yielding 0.21 gr of intermediate.

By analogous procedure the following compounds can be prepared:

- 5  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-aminoimidazole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, dihydrochloride;
- $\beta$ -[1-methyl-3-[1-methyl-3-[2-aminothiazole-4-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine,
- 10 dihydrochloride;
- $\beta$ -[1-methyl-3-[1-methyl-3-[3-amino-1,2,4, triazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, dihydrochloride.

15 **Step three** - The title compound

0.078 g of sodium bicarbonate was added to a solution of 0.21 g of intermediate in 10 ml of mixture dioxane-water (1:1).

- The mixture was cooled to 0°C and added in small portions of a
- 20 solution of 0.174 g of 4-N,N-bis(2-chloroethyl) aminobenzoyl chloride (prepared as reported in J.Org.Chem.26,4996-4997,1961) in 10 ml of dioxane.

- The reaction mixture was stirred one hour at room temperature and brought to pH 4-5 with 10% hydrochloric acid. The solvent
- 25 was evaporated in vacuo to yield a solid residue which was purified by HPLC using as eluant a mixture of acetonitrile-water-trifluoroacetic acid (10:90:0.1).

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0.26 g of the title compound was obtained.

FAB-MS:  $m/z$  699, (40,  $[M+H]^+$ ); 244, (100); 733, (70,  $[M+HCl-H]^+$ );  
661, (72,  $[M-HCl-H]^+$ )

5 m.p. 108-110°C

U.V. (EtOH 95%):  $\lambda$  max 309.55  $\epsilon=42172$ ;  $\lambda$  max 228.05  $\epsilon=31353$

PMR(DMSO- $d_6$ )  $\delta$ : 11.12 (s, 1H), 10.52 (s, 1H), 10.04 (s, 1H),  
8.79 (t,  $J=5.8$  Hz, 1H), 9.0 (b.s., 2H), 8.64 (b.s., 2H), 7.85  
(m, 2H), 7.52 (s, 1H), 7.29 (s, 1H), 7.39 (d,  $J=1.9$  Hz, 1H), 7.13  
10 (d,  $J=1.9$  Hz, 1H), 6.81 (m, 2H), 4.03 (s, 3H), 4.00 (s, 3H), 3.86  
(s, 3H), 3.6-4.0 (m, 8H), 3.53 (m, 2H), 2.71 (m, 2H).

By analogous procedure the following compounds can be prepared:

$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)  
15 amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrazole-5-  
carboxamido]pyrazole-5-carboxamido]propionamidine,  
hydrochloride (Compound no. 11);

$\beta$ -[1-methyl-3-[1-methyl-3-[2-[4-N,N-bis(2-chloroethyl)amino-  
benzene-1-carboxamido]thiazole-4-carboxamido]pyrazole-5-  
20 carboxamido]pyrazole-5-carboxamido]propionamidine,  
hydrochloride (Compound no. 64);

$\beta$ -[1-methyl-3-[1-methyl-3-[3-[4-N,N-bis(2-chloroethyl)amino-  
benzene-1-carboxamido]1,2,4-triazole-2-carboxamido]pyrazole-5-  
carboxamido]pyrazole-5-carboxamido]propionamidine,  
25 hydrochloride (Compound no. 12);



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$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride;

- 5  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[3-methoxy-4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride.

10 **Example 4**

$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 14)

- 15 To a solution of 0.29 g of 1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxylic acid (prepared as in Example 1) in 50 ml of dioxane was added 1 ml of thionyl chloride. The mixture was refluxed for 30 minutes and the solvent was evaporated in vacuo. The solid residue
- 20 obtained was dissolved in 10 ml of dry dioxane and added slowly to a solution of 0.2 g of  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-aminopyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, dihydrochloride (prepared as in Example 3) and 0.8 gr of sodium bicarbonate in 20 ml of water.
- 25 The mixture was stirred at room temperature for two hours and brought to pH 4-5 with 10% hydrochloric acid. After evaporation

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of solvent a solid residue was obtained which was purified by HPLC using as eluant a mixture of acetonitrile-water-trifluoroacetic acid (10-90-0.1), yielding 0.2 g of the title compound.

5

FAB-MS: m/z 822, (16, [M+H]<sup>+</sup>)

m.p. 220-223°C

PMR(DMSO-d<sub>6</sub>) δ : 11.12 (s, 1H), 10.64 (s, 1H), 10.58 (s, 1H),  
10.48 (s, 1H), 8.78 (t, J= 5.8Hz, 1H), 9.0 (b.s., 2H), 8.6  
10 (b.s., 2H), 7.93 (m, 2H), 7.53 (s, 1H), 7.46 (s, 1H), 7.29 (s, 1H),  
7.40 (d, J= 1.8Hz, 1H), 7.18 (d, J= 1.8Hz, 1H), 6.81 (m, 2H), 4.05  
(s, 3H), 4.04 (s, 3H), 4.01 (s, 3H), 3.87 (s, 3H), 3.7-3.9 (m, 8H),  
3.53 (m, 2H), 2.61 (m, 2H).

15 By analogous procedure the following compounds can be prepared:

β-[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound

20 no. 30);

β-[1-methyl-3-[1-methyl-3-[1-methyl-4-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]1,2,4-triazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound

25 no. 31);

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$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidinium, hydrochloride (Compound no. 32).

5

**Example 5**

$\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidinium, hydrochloride

10 (Compound no. 3)

*Step one* - The intermediate  $\beta$ -[1-methyl-3-[1-methyl-4-nitropyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidinium, hydrochloride.

15

0.88 g of 1-methyl-4-nitropyrrole-2-carboxyl chloride (prepared as reported in Tetrahedron 34,2389-2391,1978) was added in small portions to a solution of 1.06 g of N-(2'-cyanoethyl)-3-amino-1-methylpyrazole-5-carboxamide (prepared as in Anti  
20 Cancer Drug Des. 6,501-517,1991) and 0.72 ml of triethylamine in 20 ml of dioxane. The mixture was stirred overnight, concentrated to small volume, diluted with 30 ml of 5% hydrochloride acid and extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulphate and  
25 after evaporation of the solvent the solid residue was purified by recrystallization from ethyl acetate-hexane, yielding 0.8 g

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of  $\beta$ -[1-methyl-3-[1-methyl-4-nitropyrrole-2-carboxamido]pyrazole-5-carboxamido]propionitrile. 0.8 g of derivative was suspended in anhydrous ethanol and the solution saturated with dry HCl gas. After 24 h at room temperature, the solvent was  
5 evaporated in vacuo and the residue treated with ammonia in ethanol. After 24 h at room temperature the solvent was evaporated in vacuo and the solid residue purified by recrystallization from ethanol absolute yielding 0.79 g of intermediate.

10

m.p. 180-183°C

PMR(DMSO- $d_6$ )  $\delta$ : 9.42 (s,1H), 8.99 (b.s.,2H), 8.92 (b.s.,1H),  
8.68 (b.s.,2H), 8.22 (d,J= 1.8HZ,1H), 7.80 (d,J= 1.8HZ,1H),  
7.29 (s,1H), 4.01 (s,3H), 3.95 (s,3H), 3.52 (t,J= 6Hz,2H), 2.62  
15 (t,J= 6Hz,2H).

*Step two* - The title compound

0.30 g of intermediate was dissolved in a mixture of methanol-  
20 dioxane-10% hydrochloride acid (4:1:1) and reduced over Pd catalyst (10% on chorcoal) under hydrogen pressure (50 psi) in a Parr apparatus. The solution obtained after filtration of the catalyst was evaporated in vacuo, the solid residue obtained was dissolved in 5 ml of dry dimethylformamide and added of  
25 0.29 g of 1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxylic acid (prepared as in Example 1), 0.2 g of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide

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hydrochloride and 0.13 ml of N,N'-diisopropylethylamine. The mixture was stirred overnight at room temperature and brought to pH 4-5 with 10% hydrochloric acid. After evaporation of solvent the solid residue was purified by HPLC using as eluant  
5 a mixture of acetonitrile-water-trifluoroacetic acid (10-90-0.1), yielding 0.27 g of the title compound.

FAB-MS: m/z 698, (40, [M+H]<sup>+</sup>); 696, (10, [M-H]<sup>-</sup>); 660, (5, [M-HCl-H]<sup>-</sup>);  
732 (10, [M+HCl-H]<sup>-</sup>)

10 m.p. 206-210°C

U.V. (EtOH 95%): λ max 312.85, ε=39690; λ max 228.70, ε=28388

PMR(DMSO-d<sub>6</sub>) δ: 10.56 (s, 1H), 10.03 (s, 1H), 9.97 (s, 1H), 8.75  
(t, J= 5.9Hz, 1H), 9.0 (b.s., 2H), 8.6 (b.s., 2H), 7.84 (m, 2H),  
7.26 (s, 1H), 7.36 (d, J= 1.8Hz, 1H), 7.30 (d, J= 1.8Hz, 1H), 7.12  
15 (d, J= 1.8Hz, 1H), 7.08 (d, J= 1.8Hz, 1H), 6.82 (m, 2H), 3.99  
(s, 3H), 3.84 (s, 6H), 3.6-3.9 (m, 8H), 3.53 (m, 2H), 2.61 (m, 2H).

By analogous procedure and using the opportune starting material the following compounds can be prepared:

20 β-[1-methyl-3-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)  
amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-  
2-carboxamido]pyrazole-5-carboxamido]propionamide,  
hydrochloride (Compound no. 33);

25 β-[1-methyl-3-[1-methyl-4-[2-[4-N,N-bis(2-chloroethyl) amino-  
benzene-1-carboxamido]thiazole-4-carboxamido]pyrrole-2-

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carboxamido]pyrazole-5-carboxamido]propionamidine,  
hydrochloride (Compound no. 5)

FAB-MS: m/z 702, (60, [M+H]<sup>+</sup>); 244, (100); 738, (15)

PMR(DMSO-d<sub>6</sub>) δ: 12.50 (s, 1H), 10.57 (s, 1H), 9.75 (s, 1H), 9.0  
5 (b.s., 2H), 8.77 (m, 1H), 8.7 (b.s., 2H), 8.03 (m, 2H), 7.91  
(s, 1H), 7.28 (s, 1H), 7.43 (d, J=1.8 Hz, 1H), 7.13 (d, J=1.8  
Hz, 1H), 6.87 (m, 2H), 4.0 (s, 3H), 3.87 (s, 3H), 3.6-3.9 (m, 8H),  
3.54 (m, 2H), 2.61 (m, 2H).

10 β-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)  
amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrrole-2-  
carboxamido]pyrazole-5-carboxamido]propionamidine,  
hydrochloride (Compound no. 34);

β-[1-methyl-3-[1-methyl-4-[3-[4-N,N-bis(2-chloroethyl)amino-  
15 benzene-1-carboxamido]1,2,4-triazole-5-carboxamido]pyrrole-2-  
carboxamido]pyrazole-5-carboxamido]propionamidine,  
hydrochloride (Compound no. 35);

β-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-bromoethyl)  
amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-  
20 carboxamido]pyrazole-5-carboxamido]propionamidine, hydrobromide  
(Compound no. 74).

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Example 6

$\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine,hydrochloride (Compound no. 36)

*Step one* - The intermediate  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-aminopyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, dihydrochloride.

10

A solution of 0.57 g of 1-methyl-4-nitropyrrole-2-carboxyl chloride (prepared as reported in Tetrahedron 34,2389,1978) in 10 ml of dioxane was added slowly to a solution of 0.29 g of  $\beta$ -[1-methyl-3-[1-methyl-4-aminopyrrole-2-carboxamido] pyrazole-5-carboxamido]propionamidine,dihydrochloride [prepared as in Example 5] and 0.2 g of sodium bicarbonate in 10 ml of water. The mixture was stirred for two hours, the solvent was evaporated in vacuo and the solid residue chromatographed on silica gel eluting with a mixture of methylene chloride-methanol (80:20 by volume) to yield 0.27 g of  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-nitropyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride.

The derivative (0.27 g) was dissolved in a mixture of methanol-dioxane-10% hydrochloric acid (4:1:1) and reduced over Pd catalyst (10% on charcoal) under hydrogen pressure (50 psi) in

25

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a Parr apparatus. The solution obtained after filtration of the catalyst was evaporated in vacuo and the solid residue used in the next step without purification.

5 **Step two** - The title compound

A solution of 0.2 g of 1-methyl-3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrazole-5-carboxylic acid (prepared as in Example 1) in 20 ml of dry dimethylformamide  
10 was added 0.27 g of intermediate, 0.17 ml of N,N'-diisopropylethylamine and 0.13 g of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride. The mixture was stirred at room temperature overnight and brought to pH 4-5 with 10% hydrochloric acid.

15 After evaporation of solvent a solid residue was obtained which was purified by HPLC using as eluant a mixture of acetonitrile-water-trifluoroacetic acid (10-90-0.1), yielding 0.26 g of the title compound.

20 **FAB-MS:** m/z 821, (20, [M+H]<sup>+</sup>)

**PMR(DMSO-d<sub>6</sub>)**  $\delta$ : 10.91 (s, 1H), 10.64 (s, 1H), 10.48 (s, 1H), 10.01 (s, 1H), 9.0 (b.s., 2H), 8.72 (t, J= 5.8Hz, 1H), 8.65 (b.s., 2H), 7.93 (m, 2H), 7.46 (s, 1H), 7.40 (d, J= 1.8Hz, 1H), 7.24 (d, J= 1.8Hz, 1H), 7.29 (s, 1H), 7.18 (d, J= 1.8Hz, 1H), 7.11 (d, J= 1.8Hz, 1H), 6.81 (m, 2H), 4.05 (s, 3H), 4.03 (s, 3H), 3.86 (s, 3H),  
25 3.84 (s, 3H), 3.7-3.9 (m, 8H), 3.52 (m, 2H), 2.62 (m, 2H).



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By analogous procedure the following compounds can be prepared:

- $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 37);
- $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 38);
- $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 39).

#### Example 7

- $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 40)

- Step one* - The intermediate  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-aminopyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, dihydrochloride.

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To a solution of 0.2 g of 3-(benzyloxycarbonyl)amino-1-methylpyrazole-5-carboxylic acid (prepared as reported in J.Org.Chem. 54,431-434,1989) in 20 ml of dry dimethylformamide was added 0.2 g of  $\beta$ -[1-methyl-3-[1-methyl-4-aminopyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, dihydrochloride [prepared as in Example 5], 0.2 ml triethylamine and 0.14 g of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride.

The mixture was stirred overnight, the solvent was evaporated in vacuo and the solid residue chromatographed on silica gel eluting with a mixture methylene chloride-methanol (80:20 by volume), yielding 0.28 g of  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-(benzyloxycarbonyl)aminopyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride.

The derivative (0.28 g) was dissolved in a mixture of methanol-dioxane-10% hydrochloric acid (4:1:1) and reduced over Pd catalyst (10% on charcoal) under hydrogen pressure (50 psi) in a Parr apparatus. The solution obtained after filtration of the catalyst was evaporated in vacuo and the solid residue used in the next step without purification.

**Step two** - The title compound

A solution of 0.2 g of 1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxylic acid (prepared as in Example 1) in 20 ml of dry dimethylformamide

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was added 0.27 g of intermediate, 0.17 ml of N,N'-diisopropylethylamine and 0.18 g of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride. The mixture was stirred at room temperature overnight and brought to pH 4-5 with 10% hydrochloric acid.

After evaporation of solvent a solid residue was obtained which was purified by HPLC using as eluant a mixture of acetonitrile-water-trifluoroacetic acid (10-90-0.1), yielding 0.26 g of the title compound.

10

FAB-MS: m/z 822, (23, [M+H]<sup>+</sup>)

PMR(DMSO-d<sub>6</sub>) δ: 11.1 (s, 1H), 10.5 (s, 1H), 10.3 (s, 1H), 10.02 (s, 1H), 8.96 (b.s., 2H), 8.8 (t, J= 5.8Hz, 1H), 8.65 (b.s., 2H), 7.94 (m, 2H), 7.53 (s, 1H), 7.42 (s, 1H), 7.37 (s, 1H), 7.31 (d, J= 1.8Hz, 1H), 7.24 (d, J= 1.8Hz, 1H), 6.82 (m, 2H), 4.04 (s, 3H), 4.02 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.6-3.9 (m, 8H), 3.52 (m, 2H), 2.6 (m, 2H).

By analogous procedure the following compounds can be prepared:

20 β-[1-methyl-3-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidinium, hydrochloride (Compound no. 41);

25 β-[1-methyl-3-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]

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pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 42);

5  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 43).

#### Example 8

10  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 7)

15 *Step one* - The intermediate 1-methyl-3-nitropyrazole-5-carboxylic acid.

A solution of 0.8 g of methyl 1-methyl-3-aminopyrazole-5-carboxylate (prepared as reported in J.Org.Chem. 54,431-434, 20 1989) in 15 ml of chloroform was added in small portions to a solution of 1.24 g of 3-chloroperbenzoic acid in 30 ml of chloroform and refluxed for two hours. The mixture was cooled, the solid residue filtered, the solution washed for three time with 10% sodium hydroxide and dried over anhydrous sodium 25 sulphate.

After evaporation of solvent in vacuo the solid residue was purified by recrystallization from ethyl acetate-petroleum

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ether to yield 0.5 g of methyl 1-methyl-3-nitropyrazole-5-carboxylate. The derivative (0.5 g) was dissolved in 7 ml of dioxane and added of 3 ml of 2 N potassium hydroxide. The mixture was stirred for two hours, acidified with 10%  
5 hydrochloridric acid and the solvent evaporated in vacuo. The solid residue was dissolved in ethyl acetate, washed with water and dried over anhydrous sodium sulphate. After evaporation of solvent 0.4 g of intermediate was obtained.

10 m.p. 165-167°C

PMR(DMSO-d<sub>6</sub>)  $\delta$ : 7.57 (b.s., 1H), 7.37 (s, 1H), 4.27 (s, 3H).

*Step two* - The intermediate  $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-3-nitropyrazole-5-carboxamido)pyrazole-5-carboxamido)  
15 pyrazole-5-carboxamido)propionitrile.

To a solution of 0.2 g of intermediate and 0.412 g of  $\beta$ -[1-methyl-3-[1-methyl-3-aminopyrazole-5-carboxamido]pyrazole-5-carboxamido]propionitrile (prepared as reported in Anti Cancer  
20 Drug Des. 6, 501-517, 1991) in 30 ml of dry dimethylformamide was added 0.43 g of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride and 0.86 ml of N,N'-diisopropylethylamine. The mixture was stirred at room temperature overnight and the solvent was evaporated in vacuo. The solid residue obtained was  
25 dissolved in ethyl acetate and washed with solution of 10% hydrochloridic acid. The organic phase was dried over anhydrous sodium sulphate, the solvent was evaporated in vacuo to yield a

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solid which was purified by recrystallization from ethyl acetate-petroleum ether, yielding 0.323 g of intermediate.

Step three - The intermediate  $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-3-aminopyrazole-5-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine, dihydrochloride.

0.3 g of intermediate was suspended in anhydrous ethanol and the solution saturated with dry HCl gas. After 24 h at room temperature, the solvent was evaporated in vacuo and the residue treated with ammonia in ethanol. After 24 h at room temperature the solvent was evaporated in vacuo and the solid residue purified by recrystallization from ethanol absolute to yield 0.2 g of amidine derivative which was dissolved in a mixture of methanol-dioxane-10% hydrochloric acid (4:1:1) and reduced over Pd catalyst (10% on charcoal) under hydrogen pressure (50 psi) in a Parr apparatus.

The solution obtained after filtration of the catalyst was evaporated in vacuo and the solid residue purified by recrystallization from ethyl acetate-ether, yielding 0.17 g of intermediate.

PMR(DMSO-d<sub>6</sub>)  $\delta$ : 11.31 (s,1H), 11.17 (s,1H), 9.15-8.85 (bs,7H), 8.63 (b.s.,1H), 7.59 (s,1H), 7.31 (s,1H), 7.13 (s,1H), 4.09 (s,3H), 4.07 (s,3H), 4.01 (s,3H), 3.54 (t,J= 6Hz,2H), 2.73 (t,J= 6Hz,2H),

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**Step four - The title compound**

To a solution of 0.3 g of intermediate in 20 ml of water and 0.14 g of sodium bicarbonate was added slowly a solution of 0.3  
5 g of 4-N,N-bis(2-chloroethyl)aminobenzoyl chloride (prepared as in J.Org.Chem.26,4996-4997,1961) in 20 ml of dioxane.

The mixture was stirred for one hour at room temperature and brought to pH 4-5 with 10% hydrochloric acid.

After evaporation of the solvent a solid residue was obtained  
10 which was purified by HPLC using as eluant a mixture of acetonitrile-water-trifluoroacetic acid (10-90-0.1), yielding 0.18 g of the title compound.

FAB-MS: m/z 700, (20, [M+H]<sup>+</sup>)

15 U.V. (EtOH 95%):  $\lambda$  max 306.9,  $\epsilon$ =23142

PMR(DMSO-d<sub>6</sub>)  $\delta$ : 11.12 (b.s., 2H), 10.68 (s, 1H), 8.92 (t, J= 5.8 Hz, 1H), 8.64 (b.s., 4H), 7.95 (m, 2H), 7.61 (s, 1H), 7.52 (s, 1H), 7.32 (s, 1H), 6.81 (m, 2H), 4.03 (s, 3H), 4.02 (s, 3H), 4.00 (s, 3H), 3.6-4.0 (m, 8H), 3.53 (m, 2H), 2.71 (m, 2H).

20

By analogous procedure the following compound can be prepared:

$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 15).  
25

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Example 9

$\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride (Compound no. 29)

Step one - The intermediate  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-aminopyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, dihydrochloride

10

To a solution of 0.55 g of 1-methyl-3-nitropyrazole-5-carboxylic acid (prepared as in reported in Example 8) in 10 ml of benzene were added 2 ml of thionyl chloride. The mixture was refluxed for one hour and the solvent evaporated in vacuo to yield a solid residue which was dissolved in 10 ml of dioxane and added in small portions to a solution of 1 g of  $\beta$ -[1-methyl-4-[1-methyl-4-aminopyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, dihydrochloride (prepared as reported in J.Med.Chem. 32,774-778,1989) and 0.2 g of sodium bicarbonate in 10 ml of water. The mixture was stirred for one hour and then brought to pH 4-5 with 10% hydrochloric acid. The Solvent was evaporated in vacuo and the solid residue chromatographed on silica gel eluting with a mixture methylene chloride-methanol (80:20 by volume), yielding 0.7 g of  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-nitropyrazole-5-carboxamido]

25



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pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine,  
hydrochloride.

The nitro derivative (0.7 g) was dissolved in a mixture of  
methanol-dioxane-10% hydrochloric acid (4:1:1) and reduced  
5 over Pd catalyst (10% on charcoal) under hydrogen pressure (50  
psi) in a parr apparatus.

The solution obtained after filtration of the catalyst was  
evaporated in vacuo and the solid residue (0.6 g) used in the  
next step without purification.

10

*Step two* - The title compound

A solution of 0.7 g of 1-methyl-3-[4-N,N-bis(2-chloroethyl)  
amino-benzene-1-carboxamido]pyrazole-5-carboxylic acid  
15 (prepared as reported in Example 1) in 20 ml of dry  
dimethylformamide was added 0.6 g of intermediate, 0.24 ml of  
N-N'-diisopropylethylamine and 0.5 g of 1-ethyl-3-(3'-  
dimethylaminopropyl)carbodiimide hydrochloride.

The reaction mixture was stirred overnight at room temperature  
20 and brought to pH 4-5 with 10% hydrochloric acid. The Solvent  
was evaporated in vacuo to yield a solid residue which was  
purified by HPLC using as eluant a mixture of acetonitrile-  
water-trifluoroacetic acid (10:90:0.1) to yield 0.7 g of the  
title compound.

25

By analogous procedure the following compounds can be prepared:

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$\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride (Compound  
5 no. 65);

$\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride (Compound no. 66).  
10

**Example 10**

$\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride  
15 (Compound no. 67)

**Step one** - The intermediate  $\beta$ -[1-methyl-4-[1-methyl-3-nitropyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrochloride.  
20

A solution of 0.89 g of 1-methyl-3-nitropyrazole-2-carboxyl chloride (prepared as reported in Example 8) in 10 ml of dioxane was added in small portions to a solution of 1 g of 3-(1-methyl-4-aminopyrrole-2-carboxamido)propionitrile (prepared  
25 as reported in J.Org.Chem. 55,728-737,1990) and 0.95 ml of triethylamine in 10 ml of dioxane.

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The mixture was stirred overnight, concentrated to small volume, diluted with 30 ml of 5% hydrochloride acid and extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulphate and after evaporation of the solvent the solid residue was purified by recrystallization from ethyl acetate-hexane, yielding 0.9 g of  $\beta$ -[1-methyl-4-[1-methyl-3-nitropyrazole-5-carboxamido]pyrrole-2-carboxamido] propionitrile. 0.9 g of derivative was suspended in anhydrous ethanol and the solution saturated with dry HCl gas. After 24 h at room temperature, the solvent was evaporated in vacuo and the residue treated with ammonia in ethanol. After 24 h at room temperature the solvent was evaporated in vacuo and the solid residue purified by recrystallization from ethanol absolute yielding 0.8 g of intermediate.

15

**Step two - The title compound**

0.50 g of intermediate was dissolved in a mixture of methanol-dioxane-10% hydrochloride acid (4:1:1) and reduced over Pd catalyst (10% on chorcoal) under hydrogen pressure (50 psi) in a parr apparatus. The solution obtained after filtration of the catalyst was evaporated in vacuo, the solid residue obtained was dissolved in 5 ml of dry dimethylformamide and added of 0.48 g of 1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxylic acid (prepared as in Example 8), 0.48 g of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride and 0.21 ml of N,N'-diisopropylethylamine. The

25

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mixture was stirred overnight at room temperature and brought to pH 4-5 with 10% hydrochloric acid. After evaporation of solvent the solid residue was purified by HPLC using as eluant a mixture of acetonitrile-water-trifluoroacetic acid (10-90-0.1), yielding 0.4 g of the title compound.

By analogous procedure the following compounds can be prepared:

$\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine,hydrochloride  
(Compound no. 68);

$\beta$ -[1-methyl-4-[1-methyl-3-[3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine,hydrochloride  
(Compound no. 10);

$\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine,hydrochloride  
(Compound no. 9).

#### Example 11

$\beta$ -(1-methyl-4-(1-methyl-4-(2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine,hydrochloride (Compound no. 16)

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*Step one* - The intermediate 2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxylic acid.

To a solution containing 0.688 g of ethyl 2-aminothiazole-4-carboxylate (prepared as reported in J.A.C.S. 68,266,1946) and 0.3 g of 2-bromoacrylic acid in 10 ml of dioxane, 0.412 g of N-N'dicyclohexylcarbodiimide were and the mixture was stirred at room temperature overnight. After filtration, the solvent was evaporated in vacuo, the solid residue was dissolved in 50 ml of ethyle acetate, treated with saturated solution of sodium bicarbonate and then with 10% hydrochloric acid. The organic phase was dried over anhydrous sodium sulphate and the solvent evaporated in vacuo. The solid residue was purified by recrystallization from ethanol-water to yield 0.52 g of ethyl 2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxylate.

The derivative (0.52 g) was dissolved in 10 ml of dioxane and added of 1.6 ml of 2 N potassium hydroxide, the mixture was stirred overnight, acidified with 10% hydrochloric acid and the solvent was evaporated in vacuo yielding 0.45 g of intermediate.

m.p. 227-229°C

PMR(DMSO-d<sub>6</sub>)  $\delta$ : 3.04 (b.s., 1H), 8.15 (s, 1H), 8.06 (s, 1H), 7.06 (d, J= 3.7Hz, 1H), 6.54 (d, J= 3.7Hz, 1H).

25

By analogous procedure the following compounds can be prepared:

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1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxylic acid

PMR(DMSO- $d_6$ )  $\delta$ : 12.2 (b.s., 1H), 10.2 (s, 1H), 7.38 (d, J=1.8 Hz, 1H), 6.85 (d, J=1.8 Hz, 1H), 6.68 (d, J=3.7 Hz, 1H), 6.2 (d, J=3.7 Hz, 1H), 3.82 (s, 3H).

5

1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxylic acid

PMR(DMSO- $d_6$ )  $\delta$ : 12.9 (b.s., 1H), 10.1 (s, 1H), 7.22 (s, 1H), 6.95 (d, J=3.7 Hz, 1H), 6.43 (d, J=3.7 Hz, 1H), 4.02 (s, 3H).

- 10 1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxylic acid;  
3-( $\alpha$ -bromoacrylamido)1,2,4, triazole-5-carboxylic acid.

*Step two - The title compound*

- 15 To a solution containing 0.21 g of  $\beta$ -[1-methyl-4-[1-methyl-4-aminopyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine, dihydrochloride (prepared as reported in J. Med. Chem. 32,774-778,1989) in 10 ml of dry dimethylformamide, 0.15 g of intermediate, 0.153 g of 1-ethyl-3-  
20 (3'-dimethylaminopropyl)carbodiimide hydrochloride and 0.09 ml of N,N'-diisopropylethylamine were added.

The mixture was stirred overnight at room temperature and brought to pH 4-5 with 10% hydrochloric acid.

- After evaporation of the solvent a solid residue was obtained  
25 which was purified by HPLC using as eluant a mixture of

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acetonitrile-water-trifluoroacetic acid (10-90-0.1), yielding 0.13 g of the title compound.

FAB-MS: m/z 592, (40, [M+H]<sup>+</sup>)

5 PMR(DMSO-d<sub>6</sub>) δ: 10.00 (s, 1H), 9.88 (s, 1H), 8.98 (b.s., 2H), 8.63 (b.s., 2H), 8.23 (t, J= 5.8Hz, 1H), 7.99 (s, 1H), 7.28 (d, J= 1.8 Hz, 1H), 7.20 (d, J= 1.8Hz, 1H), 7.10 (d, J= 1.8Hz, 1H), 7.04 (d, J= 3.7Hz, 1H), 6.95 (d, J= 1.8Hz, 1H), 6.54 (d, J= 3.7Hz, 1H), 3.85 (s, 3H), 3.8 (s, 3H), 3.48 (m, 2H), 2.61 (m, 2H).

10

By analogous procedure and using opportune intermediate prepared as described above, the following compounds can be prepared:

β-(1-methyl-4-(1-methyl-4-(1-methyl-3-(α-bromoacrylamido)

15 pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 17)

FAB-MS: m/z 587, (8, [M+H]<sup>+</sup>)

PMR(DMSO-d<sub>6</sub>) δ: 11.03 (s, 1H), 10.48 (s, 1H), 9.98 (s, 1H), 9.00 (b.s., 2H), 8.6 (b.s., 2H), 8.22 (t, J= 5.8Hz, 1H), 7.35 (s, 1H), 20 7.3 (d, J= 1.8Hz, 1H), 7.19 (d, J= 1.8Hz, 1H), 7.08 (d, J= 1.8Hz, 1H), 6.94 (d, J= 1.8Hz, 1H), 6.80 (d, J= 3.7Hz, 1H), 6.32 (d, J= 3.7Hz, 1H), 4.04 (s, 3H), 3.85 (s, 3H), 3.8 (s, 3H), 3.5 (m, 2H), 2.6 (m, 2H).

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$\beta$ -(1-methyl-4-(1-methyl-4-(3-( $\alpha$ -bromoacrylamido)1,2,4, triazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido) propionamidine, hydrochloride (Compound no. 18);

$\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 22);

$\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-4-(1-methyl-3( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 28)

FAB-MS: m/z 709, (7, [M+H]<sup>+</sup>)

PMR(DMSO-d<sub>6</sub>)  $\delta$ : 11.02 (s, 1H), 10.48 (s, 1H), 10.00 (s, 1H), 9.92 (s, 1H), 8.9 (b.s., 2H), 8.5 (b.s., 2H), 8.21 (m, 1H), 7.35 (s, 1H), 7.30 (d, J= 1.8Hz, 1H), 7.24 (d, J= 1.8Hz, 1H), 7.17 (d, J= 1.8Hz, 1H), 7.09 (d, J= 1.8Hz, 1H), 7.06 (d, J= 1.8Hz, 1H), 6.95 (d, J= 1.8Hz, 1H), 6.79 (d, J= 3.4Hz, 1H), 6.31 (d, J= 3.4Hz, 1H), 4.04 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.8 (s, 3H), 3.49 (m, 2H), 2.59 (m, 2H).

20

$\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-4(2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 44);



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$\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-4-(3-( $\alpha$ -bromoacrylamido)1,2,4, triazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 45);

5

$\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 46)

10 FAB-MS: m/z 709, (15, [M+H]<sup>+</sup>); 256

U.V. (EtOH 95%):  $\lambda$  max 312.85,  $\epsilon$ =41902;  $\lambda$  max 239.9,  $\epsilon$ =24543

PMR(DMSO-d<sub>6</sub>)  $\delta$ : 10.09 (s, 1H), 10.06 (b.s., 1H), 9.99 (s, 1H), 9.91 (s, 1H), 8.9 (b.s., 4H), 8.23 (t, J=5.9 Hz, 1H), 7.52 (s, 1H), 7.26 (d, J=1.8Hz, 1H), 7.23 (d, J=1.8Hz, 1H), 7.17 (d, J=1.8Hz, 1H), 15 7.16 (d, J=1.8Hz, 1H), 7.06 (d, J=1.8Hz, 1H), 6.93 (d, J=1.8Hz, 1H), 6.80 (d, J=3.1Hz, 1H), 6.29 (d, J=3.1Hz, 1H), 3.97 (s, 3H), 3.84 (s, H), 3.83 (s, 3H), 3.79 (s, 3H), 3.49 (m, 2H), 2.61 (t, J=6.2Hz, 2H).

$\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride (Compound no. 19);

$\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 47);

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- $\beta$ -(1-methyl-3-(1-methyl-3-(2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 48);
- $\beta$ -(1-methyl-3-(1-methyl-3-(3-( $\alpha$ -bromoacrylamido)1,2,4, triazole-5-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 49);
- $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 23);
- 10  $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-4-(1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 50);
- $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 51);
- 15  $\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 52);
- 20  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 20);

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- $\beta$ -(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 69);
- $\beta$ -(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 24);
- $\beta$ -(1-methyl-4-(1-methyl-3-(1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 70);
- 10  $\beta$ -(1-methyl-3-(1-methyl-4-(3-( $\alpha$ -bromoacrylamido)1,2,4-triazole-5-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 25);
- $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 53);
- 15  $\beta$ -(1-methyl-3-(1-methyl-4-(2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 21);
- $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 54);
- 20  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-(1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine,
- 25 hydrochloride (Compound no. 55);

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- $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 56);
- 5  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-(3-( $\alpha$ -bromoacrylamido)1,2,4, triazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 57);
- 10  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-4-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 58);
- 15  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-3-(1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 59);
- 20  $\beta$ -(1-methyl-3-(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)propionamidine, hydrochloride (Compound no. 60);
- 25  $\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-3-(1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine, hydrochloride (Compound no. 26);
- 25  $\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)

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pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine,  
hydrochloride (Compound no. 71);

$\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -  
bromoacrylamido)imidazole-2-carboxamido)pyrazole-5-carboxamido)  
5 pyrrole-2-carboxamido)pyrrole-2-carboxamido)propionamidine,  
hydrochloride (Compound no. 72);

$\beta$ -(1-methyl-3-(1-methyl-3-(1-methyl-3-(1-methyl-3-( $\alpha$ -  
bromoacrylamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)  
pyrazole-5-carboxamido)pyrazole-5-carboxamido)propionamidine,  
10 hydrochloride (Compound no. 27).

#### Example 12

$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-bromoethyl)  
amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-  
15 carboxamido]pyrazole-5-carboxamido]propionamidine, hydrobromide  
(Compound no. 75)

*Step one* - The intermediate 4-N,N-bis-(2-bromoethyl)amino-1-(1-  
imidazolylcarbonyl)benzene.

20

To a solution of 1.5 g of ethyl 4-N,N-bis-(2-bromoethyl)amino-  
benzoic acid (prepared as reported in J.Med.Chem. 21,16,1978)  
in 50 ml of ethyl acetate, were added 850 mg of N-  
N'carbonyldiimidazol. The solution was stirred at room  
25 temperature for 4 hours. The solvent was partially removed in  
vacuo to afford the intermediate as a white precipitate.

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m.p. (EtOAc) 104-106°C

PMR(CDCl<sub>3</sub>)  $\delta$  : 8.07 (s,1H), 7.76 (m,2H), 7.5 (m,1H), 7.13 (m,1H), 6.73 (m,2H), 3.88 (t,J=7.4Hz,4H), 3.49 (t,J=7.4Hz,4H).

5 By analogous procedure and using the opportune starting material the following compounds can be prepared:

1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]-2-(1-imidazolylcarbonyl)pyrrole;

PMR(CDCl<sub>3</sub>)  $\delta$  : 8.78 (s,1H), 8.22 (s,1H), 7.86 (m,1H), 7.83  
10 (m,2H), 7.58 (m,1H), 7.11 (d,J=1.8Hz,1H), 6.75 (d,J=1.8Hz,1H),  
6.66 (m,2H), 3.96 (s,1H), 3.82 (t,J=7.3Hz,4H), 3.45  
(t,J=7.3Hz,4H).

1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-  
15 carboxamido]-5-(1-imidazolylcarbonyl)pyrazole.

*Step two* - The title compound

The intermediate  $\beta$ -[1-methyl-3-[1-methyl-3[1-methyl-4-amino-  
20 pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-  
carboxamido]propionamidine, dihydrochloride (prepared as  
reported in Step two of Example 3) was transformed into the  
corresponding dihydrobromide using a strong basic anion  
exchange resin. The intermediate dihydrobromide (1.02 gr, 1.66  
25 mmole) was dissolved in 20 ml of dry dimethylformamide and 1 gr  
(2.5 mmole) of 4-N,N-bis(2-bromoethyl)amino-1-(1-  
imidazolylcarbonyl)benzene was added. The solution was stirred

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for 4 hours at 50°C, the solvent removed in vacuo and the residue purified by flash chromatography (methylenchloride-methanol 8:2) to yield 950 mg of the title compound.

5 m.p. 210-211°C

FAB-MS: m/z 787, (20, [M+H]<sup>+</sup>); 332 (30)

PMR(DMSO-d<sub>6</sub>) δ: 11.17 (s, 1H), 10.56 (s, 1H), 10.06 (s, 1H), 8.95 (s, 2H), 8.79 (bs, 1H), 8.49 (s, 2H), 7.86 (m, 2H), 7.54 (s, 1H), 7.41 (d, J=1.9Hz, 1H), 7.31 (s, 1H), 7.15 (s, J=1.9Hz, 1H), 6.81 (m, 2H), 4.05 (s, 3H), 4.01 (s, 3H), 4.02 (s, 3H), 3.88 (m, 4H), 3.63 (m, 4H), 3.42 (m, 2H), 2.6 (m, 2H).

10

By analogous procedure and using opportune intermediate prepared as described above, the following compounds can be prepared:

15

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine, hydrobromide (Compound no. 76);

20 β-[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrobromide (Compound no. 77);

β-[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]

25

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pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrobromide (Compound no. 78);  
 $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrobromide (Compound no. 79);  
 $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrobromide (Compound no. 80);  
 $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrobromide (Compound no. 81).

15

**Example 13**

Intramuscular injection 10 mg/ml

An injectable pharmaceutical composition can be manufactured by dissolving 10 g of  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine, hydrochloride in water for injection (1000 ml) and sealing ampoules of 1-5 ml.



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**Example 14**

Capsules, each dosed at 0.200 g and containing 10 mg of the active substance can be prepared as follows:

Composition for 500 capsules:

5	$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis (2-chloroethyl) amino-benzene-1-carboxamido]pyrrole-4- carboxamido]pyrazole-5-carboxamido]pyrazole-5- carboxamido]propionamidine hydrochloride	5 g
	Lactose	85 g
10	Corn starch	5 g
	Magnesium stearate	5 g

This formulation can be encapsulated in two-piece hard gelatin capsules and dosed at 0.200 g for each capsule.

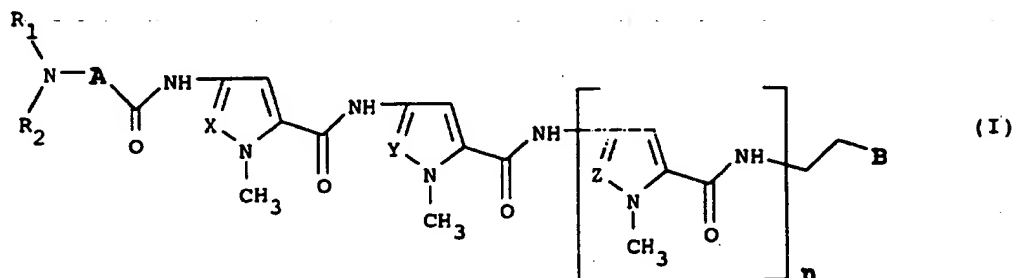
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**CLAIMS**

1. A compound of formula (I)

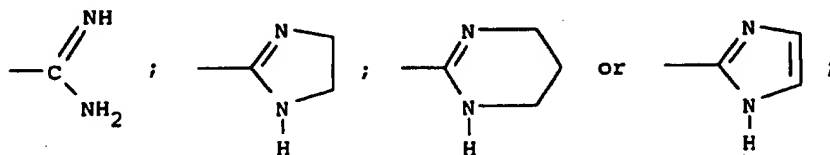


5 wherein n is 0 or 1;

each of X, Y, Z is independently N or CH;

A is a pentatomic heteromonocyclic ring unsubstituted or substituted by a C<sub>1</sub>-C<sub>3</sub> alkyl group;

B is



10

and either R<sub>1</sub> and R<sub>2</sub> are the same and they are both a C<sub>1</sub>-C<sub>6</sub> alkyl group unsubstituted or substituted by halogen or hydroxy; or one of R<sub>1</sub> and R<sub>2</sub> is hydrogen and the other is a group -C-(CH<sub>2</sub>)<sub>m</sub>-R<sub>4</sub> in which m is zero or an integer of 1

15



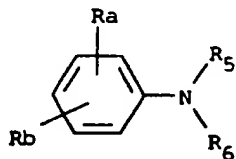
to 4 and R<sub>4</sub> is aziridinyl; cyclopropyl; a vinyl group unsubstituted or substituted in position 1 or 2 by halogen or C<sub>1</sub>-C<sub>3</sub> alkyl; an oxyranyl group unsubstituted or substituted in position 2 by a C<sub>1</sub>-C<sub>3</sub> alkyl or halogen; or

20

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a group in which  $R_5$  and  $R_6$  are both a  $C_1$ - $C_6$  alkyl group unsubstituted or substituted by a halogen or hydroxy; provided that:

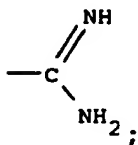
- 5 a) when each of X, Y, Z is CH, then A is not thiophene or pyrrole unsubstituted or substituted by a  $C_1$ - $C_3$  alkyl group; and
- b) when n is 1, each of X, Y, Z is CH and  $R_1$  and  $R_2$  are the same and they are both 2-chloroethyl, then A is not 1-methylimidazole or thiazole; and the pharmaceutically
- 10 acceptable salt thereof.

2. A compound of formula (I) according to claim 1, wherein:

n is 0 or 1; each of X, Y, Z independently is N or CH;

15 A is thiazole, imidazole, 1,2,4-triazole, 1-methylpyrrole, 1-methylimidazole, 1-methylpyrazole;

B is



and either  $R_1$  and  $R_2$  are the same and they are both 2-chloroethyl, 2-fluoroethyl, 2-bromoethyl or one of  $R_1$  and

20  $R_2$  is hydrogen and the other is  $\alpha$ -bromoacryloyl, 4-N,N-bis(2-chloroethyl)amino-benzene-1-carbonyl, 4-N,N-bis(2-bromoethyl)amino-benzene-1-carbonyl, provided that:

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- a) when each of X, Y, Z is CH, then A is not 1-methyl-pyrrole; and
- b) when n is 1, each of X, Y, Z is CH and R<sub>1</sub> and R<sub>2</sub> are the same and they are both 2-chloroethyl, then A is not 1-methylimidazole or thiazole, and the pharmaceutically acceptable salts thereof.
- 5
3. A compound selected from the group consisting of the following compounds:
- 10  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 15  $\beta$ -[1-methyl-4-[1-methyl-4-[2-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]thiazole-4-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 20  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;

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- $\beta$ -[1-methyl-3-[1-methyl-4-[2-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]thiazole-4-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 5  $\beta$ -[1-methyl-4-[1-methyl-4-[3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]triazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 10  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 15  $\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 20  $\beta$ -[1-methyl-4-[1-methyl-3-[3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]-1,2,4-triazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 25  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]imidazole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;

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$\beta$ -[1-methyl-3-[1-methyl-3-[3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]-1,2,4-triazole-5-carboxamido] pyrazole-5-carboxamido]pyrazole-5-carboxamido] propionamidine;

5  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

10  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;

15  $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;

$\beta$ -[1-methyl-4-[1-methyl-4-[2( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

20  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

25  $\beta$ -[1-methyl-4-[1-methyl-4-[3-( $\alpha$ -bromoacrylamido)-1,2,4-triazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

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- $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 5  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- $\beta$ -[1-methyl-3-[1-methyl-4-[2-( $\alpha$ -bromoacrylamido)thiazole-4-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 10  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- $\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;
- 15  $\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-4-( $\alpha$ -bromoacrylamido)imidazole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine;
- $\beta$ -[1-methyl-4-[1-methyl-3-[3-( $\alpha$ -bromoacrylamido)-1,2,4-triazole-5-carboxamido]pyrrole-5-carboxamido]pyrazole-2-carboxamido]propionamidine;
- 20  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 25

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$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-3-[1-methyl-3-( $\alpha$ -  
bromoacrylamido)pyrazole-5-carboxamido]pyrazole-5-  
carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]  
propionamidine;

5  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-( $\alpha$ -  
bromoacrylamido)pyrazole-5-carboxamido]pyrrole-2-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
propionamidine;

10  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-3-[4-N,N-  
bis(2-chloroethyl)amino-benzene-1-carboxamido]pyrazole-5-  
carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]  
pyrrole-2-carboxamido]propionamide;

15  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[2-[4-N,N-bis(2-  
chloroethyl)amino-benzene-1-carboxamido]thiazole-4-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrrole-2-carboxamido]propionamidine;

20  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-  
bis(2-chloroethyl)amino-benzene-1-carboxamido]imidazole-2-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrrole-2-carboxamido]propionamidine;

25  $\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-[4-N,N-bis(2-  
chloroethyl)amino-benzene-1-carboxamido]1,2,4, triazole-5-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrrole-2-carboxamido]propionamidine;

25  $\beta$ -[1-methyl-3-[1-methyl-3-[2-[4-N,N-bis(2-chloroethyl)  
amino-benzene-1-carboxamido]thiazole-4-carboxamido]



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pyrazole-5-carboxamido]pyrazole-5-carboxamido]

propionamidine;

$\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]imidazole-2-

5 carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

$\beta$ -[1-methyl-4-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrrole-2-

10 carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

$\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-3-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine;

15  $\beta$ -[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-chloroethyl) amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine;

20  $\beta$ -(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)propionamidine;

$\beta$ -(1-methyl-4-(1-methyl-3-(1-methyl-3-( $\alpha$ -bromoacrylamido)pyrazole-5-carboxamido)pyrazole-5-carboxamido)pyrrole-2-carboxamido)propionamidine;

25  $\beta$ -(1-methyl-4-(1-methyl-4-(1-methyl-3-(1-methyl-4-( $\alpha$ -bromoacrylamido)pyrrole-2-carboxamido)pyrazole-5-

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- carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)  
propionamidine;
- β-(1-methyl-4-(1-methyl-4-(1-methyl-3-(1-methyl-4-(α-  
bromoacrylamido)imidazole-2-carboxamido)pyrazole-5-  
5 carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)  
propionamidine;
- β-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-  
bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
10 propionamidine;
- β-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-  
bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]  
propionamidine;
- 15 β-[1-methyl-3-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-  
bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-  
carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]  
propionamidine;
- 20 β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-  
bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-  
carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]  
pyrrole-2-carboxamido]propionamidine;
- 25 β-[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-3-[4-N,N-  
bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-  
carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]  
pyrazole-5-carboxamido]propionamidine;

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$\beta$ -[1-methyl-3-[1-methyl-3-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrazole-5-carboxamido]propionamidine;

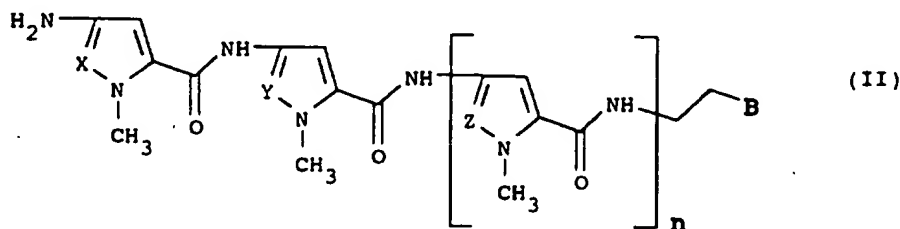
5  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-3-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;

10  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine;

15  $\beta$ -[1-methyl-3-[1-methyl-4-[1-methyl-3-[1-methyl-4-[4-N,N-bis(2-bromoethyl)amino-benzene-1-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]pyrrole-2-carboxamido]pyrazole-5-carboxamido]propionamidine, and the pharmaceutically acceptable salts thereof.

4. A process for preparing a compound of formula (I) according to claim 1, or a pharmaceutically acceptable salt thereof, comprising:

a) reacting a compound of formula (II)

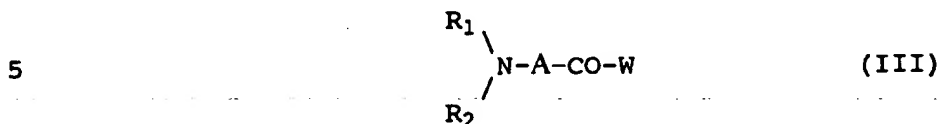


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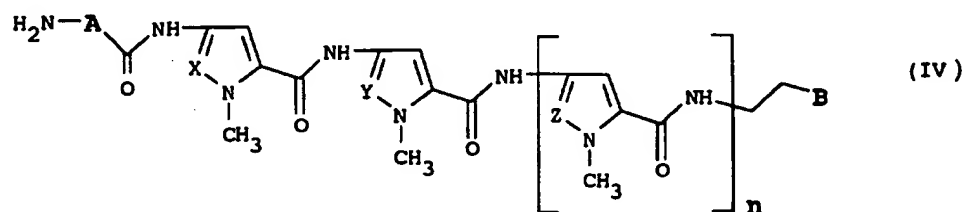
wherein X, Y, Z, B and n are as defined in claim 1 or a salt thereof with a compound of formula (III)



wherein  $R_1$ ,  $R_2$  and A are as defined in claim 1 and W is hydroxy or a leaving group; or

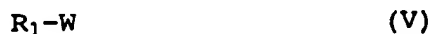
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b) reacting a compound of formula (IV)



wherein A, X, Y, Z, B and n are as defined in claim 1 or a salt thereof with a compound of formula (V)

15



wherein  $R_1$  is as defined in claim 1 and W is as defined above so obtaining a compound of formula (I) wherein  $R_1$  is as defined in claim 1 and  $R_2$  is hydrogen or is the same as  $R_1$  and, if desired salifying a compound of formula (I) or obtaining a free compound from a salt, and/or, if desired, separating a mixture of isomers of formula (I) into the single isomers.

20

25 5. A pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof,

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according to claim 1, as the active principle, and a pharmaceutically acceptable carrier and/or diluent.

- 5 6. A compound of formula (I) according to claim 1, or a pharmaceutically acceptable salt thereof, for use as antitumor or antiviral agent.
- 10 7. Use of a compound of formula (I) according to claim 1, or a pharmaceutically acceptable salt thereof, in the preparation of a medicament useful as antitumor or antiviral agent.
- 15 8. A method of treating a tumor in a patient in need of it, comprising administering to the patient an effective amount of a compound of formula (I) according to claim 1.
9. A method of treating a tumor in a patient in need of it, comprising administering to the patient an effective amount of a composition according to claim 5.

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 95/02814

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D403/14 C07D417/14 A61K31/40

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO,A,94 25436 (MENARINI INDUSTRIES) 10 November 1994 see the whole document ---	1-9
P,A	WO,A,94 20463 (BRISTOL-MYERS SQUIBB) 15 September 1994 see the whole document ---	1-9
A	EP,A,0 388 948 (CARLO ERBA) 26 September 1990 see the whole document ---	1-9
A	EP,A,0 246 868 (CARLO ERBA) 23 November 1987 see the whole document ---	1-9
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

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Date of the actual completion of the international search

31 October 1995

Date of mailing of the international search report

01.12.95

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Kissler, B

## INTERNATIONAL SEARCH REPORT

Inter- national Application No  
PCT/EP 95/02814

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE, A, 36 23 880 (CARLO ERBA) 29 January 1987 see the whole document ---	1-9
A	J. MED. CHEM., vol. 32, no. 4, 1989 pages 774 - 778 Arcamone, Federico Maria; Animati, Fabio; Barbieri, Brunella; Configliacchi, Emanuela; D'Alessio, Roberto; Geroni, Cristina; Giuli 'Synthesis, DNA-binding properties, and antitumor activity of novel distamycin derivatives' see the whole document ---	1-9
A	BIOORG. MED. CHEM., vol. 4, no. 12, 1994 pages 1467 - 1472 D'Alessio, Roberto; Geroni, Cristina; Biasoli, Giovanni; Pesenti, Enrico; Grandi, Maria; Mongelli, Nicola 'Structure-activity relationship of novel distamycin A derivatives: Synthesis and antitumor activity' see the whole document -----	1-9

**INTERNATIONAL SEARCH REPORT**

information on patent family members

International Application No  
PCT/EP 95/02814

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9425436	10-11-94	AU-B- 6646394	21-11-94
WO-A-9420463	15-09-94	AU-B- 6206894	26-09-94
EP-A-0388948	26-09-90	AU-B- 635733	01-04-93
		AU-B- 5276190	22-10-90
		DE-D- 69005164	27-01-94
		DE-T- 69005164	31-03-94
		WO-A- 9011277	04-10-90
		EP-A- 0416075	13-03-91
		ES-T- 2062143	16-12-94
		FI-B- 95463	31-10-95
		JP-T- 3504863	24-10-91
		US-A- 5175182	29-12-92
EP-A-0246868	25-11-87	AU-B- 597659	07-06-90
		AU-B- 7316387	26-11-87
		CA-A- 1314551	16-03-93
		DE-A- 3781716	22-10-92
		IE-B- 60198	15-06-94
		JP-B- 6023193	30-03-94
		JP-A- 62294653	22-12-87
		SU-A- 1528316	07-12-89
		US-A- 5017599	21-05-91
		US-A- 5049579	17-09-91
		US-A- 5310752	10-05-94
		ZA-A- 8703593	12-11-87
DE-A-3623880	29-01-87	AT-B- 387013	25-11-88
		AU-B- 587841	31-08-89
		AU-B- 6020286	22-01-87
		BE-A- 905110	15-01-87
		CH-A- 674206	15-05-90
		FR-A- 2585018	23-01-87
		GB-A, B 2178036	04-02-87
		IE-B- 59073	12-01-94
		JP-B- 7080843	30-08-95
		JP-A- 62077362	09-04-87
		KR-B- 9310496	25-10-93
		NL-A- 8601837	16-02-87



**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/EP 95/02814

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A-3623880		SE-B- 468642	22-02-93
		SE-A- 8603098	17-01-87
		SU-A- 1544185	15-02-90
		SU-A- 1609445	23-11-90
		US-A- 4766142	23-08-88
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